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OZONE AND CHLORINE INACTIVATION OF CRYPTOSPORIDIUM IN WATER

by

LYNDON LESTER GYÜRÉK

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DEDICATION

This work is dedicated to my parents, Leslie and Julia Gyürék, and to my wife, Hengameh Kharaghani, who provided me with encouragement and inspiration throughout this work.

Abstract

The disinfection efficiency of ozone and chlorine compounds on *Cryptosporidium parvum* oocysts was studied using bench-scale batch reactors and phosphate buffered laboratory water at pH 6 to 8 and 22°C. Animal infectivity using neonatal CD-1 mice was used as the criterion of oocyst viability. Inactivation data were fitted to a robust kinetic model, the Incomplete gamma Hom (I.g.H.) model, which can account for decreasing disinfectant residuals during the contact time. I.g.H. models were used to generate design graphs which can aid engineers in establishing disinfection requirements for controlling *C. parvum* in drinking water. The current approach of the Surface Water Treatment Rule to assess disinfection efficiency based on concentration × time (CT) tables may over- or underestimate disinfection requirements because of the inadequacy of the Chick-Watson model and the assumption of a constant disinfectant concentration.

The most effective disinfectant against *C. parvum* oocysts is ozone. *C. parvum* ozone disinfection data at 7° and 22°C was used to calculate an Arrhenius activation energy of 3.8 kcal/mol, the magnitude of which suggests that the ozone inactivation process is entirely controlled by mass transfer of ozone into the oocyst. Free (available) chlorine (FAC) at pH 6 and 22°C does have an effect on *C. parvum* and can provide 0.5 log-units inactivation of *C. parvum* under practical water treatment plant conditions. Synergism was used to explain the finding that chloramination practiced using post-ammoniation is more effective than free chlorine alone. Ozone followed by either free chlorine at pH 6 or monochloramine at pH 8 offers an effective treatment for controlling *C. parvum* in drinking water.

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LIST OF SYMBOLS

A	Arrhenius pre-exponential factor
a	number of CD-1 neonates used in dose-response study per given oocyst batch
C	chemical disinfectant concentration at time t, mg/L
C_c	concentration of monochloramine following conversion of free chlorine, mg/L
C_f	chemical disinfectant residual at the end of the contact time, mg/L
C_g	geometric mean (formed + preformed) monochloramine residual, mg/L
C_o	initial chemical disinfectant residual at time zero, mg/L
CT	disinfectant concentration, mg/L, × contact time, min
D	maximum absolute difference between Kaplan-Meir product-limit cumulative distribution function and standard normal distribution
E_a	Arrhenius activation energy
FAC	Free (available) chlorine, mg/L
K_a	acid dissociation constant, moles/L
k, k	inactivation rate constant
$\hat{k}$	optimal k parameter estimate
k′	first-order chemical disinfectant decay rate constant, min^{-1}
k_T	inactivation rate constant for a specified temperature
k_1	Chick reaction rate constant
k^*	pseudo first-order Chick reaction rate constant
k'	Watson's constant

L	likelihood function for normally distributed errors
L	number of organisms per clump in the Multiple series-event model
$\ln L_{max}$	global maxima for the natural logarithm of the likelihood function
l	number of lethal reactions for a single organism in the Series-event model
m	constant for the generalized inactivation rate law
$\hat{m}$	optimal m parameter estimate
N	number of surviving organisms at time t
N_o	number of organisms (assuming no clumping) at time zero
$\hat{N}_o$	optimal N_o (nuisance) parameter estimate for the Rational and Hom-Power law models
N_c	number of surviving, uniform, clumps at time t
N_{c_o}	initial number of uniform clumps at time zero
n	constant for the generalized inactivation rate law
$\hat{n}$	optimal n parameter estimate
n	estimated infectious dose per animal after disinfection
n_c	number of critical targets per particle in the Multiple-target model
n_o	the number of oocysts given to each animal, inoculum size
P	proportion of cohort positive for infection
P_t	probability of survival at time t for Multiple-target and Series-event models
P_{1-t}	probability of inactivation at time t for probabilistic models
P_i	jackknife pseudo-values for $\hat{\beta}$ for deletion of the i^{th} trial

p	number of model parameters
p	exponent of inactive microbe in Chick disinfection reaction
Q_{10}	change in activity per 10°C rise in temperature
R	universal gas constant, 1.987 cal/g-mole K
r_c	chemical disinfectant rate of decay, dC/dt
r_s	microbial rate of inactivation, dN/dt
S	survival ratio, N/N_o
T	total contact time (limit of integration), min
T_c	elapsed time between addition of ammonium chloride and preformed stock monochloramine for conversion of free chlorine to monochloramine
T	temperature in Kelvins, °K
T_o	intermediate temperature, °K
t	contact time, min
v	total number of experimental trials per data set
v_o	number of non-censored experimental trials per data set
X	oocyst inoculum given to each CD-1 neonate, log-units
x	empirical constant in the Rational and Hom-Power law models
$\hat{x}$	optimal x parameter estimate
y_i	observed survival ratio for i^{th} trial, log-units
$y_i^{\leq}$	infectivity detection limit for i^{th} trial, log-units
z	total number of Trapezoidal Rule partitions per disinfection trial

α	level of statistical significance
β	regression model parameters, (β_o, β_1)' or (k, m, n, x)'
$\bar{\beta}_J$	jackknife parameter estimates
χ^2	chi-squared distribution
Δt	Trapezoidal Rule partitions
γ	incomplete gamma function
η	efficiency factor used in analytical Hom approximation
σ	square root of the maximum likelihood regression error variance
$\hat{\sigma}$	optimal σ parameter estimate
μ_i	inactivation model predicted survival ratio for trial i, log-units
π'	logit mean response
θ	temperature coefficient
$\Phi(z)$	standard cumulative normal distribution

Subscripts

i	index of summation for experimental trials
κ	index of summation for target sites in Series-event kinetic model

Chapter 1

Introduction

1.1 Overview

Encysted waterborne parasites such as *Cryptosporidium parvum*, as well as *Giardia lamblia*, pose a threat to drinking water supplies. *C. parvum* is a coccidian protozoan that is recognized as a cause of waterborne disease in humans (Barer and Wright 1990), and has been shown to be able to cross-infect rodents, ruminants, and humans (Fayer and Ungar 1986). Oocysts, the dormant form of the protozoan, can survive prolonged periods of time in the environment and are ubiquitous in surface waters (D'Antonio et al. 1985; Gallaher et al. 1989). Ingestion of *C. parvum* oocysts can produce the disease cryptosporidiosis, a potentially life-threatening diarrhea illness in persons with underdeveloped or suppressed immune systems (Fayer and Ungar 1986). At present there is no safe and effective treatment for cryptosporidiosis. Cryptosporidiosis outbreaks from surface water supplies have been documented in Canada, the United States and Great Britain (Hayes et al. 1989; Pett et al. 1994; Rush et al. 1990).

Giardia lamblia, a human intestinal binucleate flagellated protozoan which causes prolonged diarrhea and other intestinal symptoms, is the most frequently isolated enteric protozoan from populations worldwide (Lengerich et al. 1994). Infective cysts present in potable water are considered the primary source of *Giardia* epidemics (Foliguet et al. 1987; Roach et al. 1993). *C. parvum* oocysts have been found in surface waters more frequently and in greater numbers than *Giardia lamblia* (Rose et al. 1988), and is

significantly more resistant to inactivation by chemical disinfectants than *G. lamblia* cysts (Korich et al. 1990b; Smith et al. 1988).

To safeguard against waterborne outbreaks of giardiasis, the Surface Water Treatment Rule (SWTR) requires 3 log-units removal-inactivation of *Giardia* spp. cysts from surface water supplies, and assumes that effective filtration at a conventional treatment plant will provide 2.5 log-units removal (USEPA 1990). Disinfection credit is awarded on the basis of CT products (characteristic disinfectant concentration × characteristic contact time) derived from Chick-Watson kinetics and assuming a constant disinfectant concentration. The CT concept relates the level of organism inactivation to the concentration of disinfectant and contact time as a linear function, and as such, is only meaningful if the "true" influence on the rate of microbial inactivation by disinfectant concentration and contact time are nearly equal. As well, disinfectant demand-free conditions are unlikely for most oxidants and natural waters (Hoff 1987). The limited applicability of the CT concept has important implications regarding the validity of interpreting disinfection studies based on CT values.

C. parvum is not currently regulated but inactivation-removal requirements for *C. parvum* are likely to be included in the Enhanced Surface Water Treatment Rule (ESWTR). Occurrence data for *C. parvum* in source waters collected under the Information Collection Rule (ICR) are to be used to determine the level of removal-inactivation required. A multiple barrier approach using watershed protection, pretreatment, filtration, and disinfection is necessary to prevent transmission of *C. parvum* oocysts from surface waters supplies. Given the size of oocysts, 2 to 5 μm in diameter

(Soave and Armstrong 1986), and site specific conditions (quality of raw water, plant design, operational procedures), oocysts may breach treatment filters making disinfection the last barrier to transmission of cryptosporidiosis (Hayes et al. 1989). The resistant nature of oocysts to inactivation by chemical agents requires the application of effective disinfectants in conjunction with an adequate means of assessing performance criteria to ensure an acceptable level of infectious risk and to minimize the formation of disinfection by-products.

There were few data found in the literature that investigated the effect of chemical disinfectants on *C. parvum*. Chlorine is the most widely used chemical disinfectant in the world. Elemental chlorine (Cl_2), hypochlorous acid (HOCl), and hypochlorite ion (OCl^-) are collectively referred to as free (available) chlorine (FAC). Above pH 2, HOCl and OCl^- are the major chlorine species (Snoeyink and Jenkins 1980). It has long been known that HOCl and OCl^- are the active agents in free chlorine, with HOCl being more effective (Fair et al. 1948). Early research reported that chlorine bleach solutions had little effect on oocyst viability (Campbell et al. 1982; Smith et al. 1988; Sundermann et al. 1987). These studies typically used chlorine bleach concentrations >1,000 mg/L to affect <1 log-unit inactivation. It was suggested that free chlorine was ineffective at practical treatment doses (<5 mg/L) (Korich et al. 1990a). It was later reported that 80 mg/L of free chlorine applied for 90 min provided only 1 log-unit inactivation of *C. parvum* (Korich et al. 1990b). No data could be found assessing the effect on *C. parvum* by free chlorine concentrations and contact times practical to the water treatment industry, 1 to 6 mg/L and 30 to 60 min, respectively, using animal infectivity as the viability criterion. As well,

no kinetic studies involving inactivation of *C. parvum* by chlorine compounds could be found in the literature.

The resistance of encysted protozoans has lead to increased usage of alternative chemical disinfectants. A number of research groups have investigated ozone and chlorine dioxide for inactivation of *C. parvum* under bench-scale conditions (Finch et al. 1993; Korich et al. 1990b; Parker et al. 1993; Peeters et al. 1989; Perrine et al. 1990; Ransome et al. 1993). Ozone was found to be the most effective disinfectant for controlling protozoans in drinking water. These studies used different ozonation methodologies which may influence microbial survival curves (Finch et al. 1992); animal infectivity and excystation methods for assessing oocyst viability; and different inactivation rate laws for interpretation of the data. Of these studies very few have used an experimental protocol that is amenable to rigorous analysis of the kinetics of inactivation and the simultaneous disappearance of ozone. A complete data set for ozone inactivation of encysted *C. parvum* was published by Finch et al. (1993).

There have been reports in the literature of synergism between disinfectant compounds leading to more effective treatment. Synergism is demonstrated when disinfectants applied simultaneously (or sequentially) provide greater inactivation than expected from the additive effects of the single disinfectants applied alone. Most studies have reported results from a mixture of chemical disinfectants used simultaneously (Kouame and Haas 1991; Venczel et al. 1996). Because free chlorine or monochloramine are frequently added as the final disinfectant in water treatment facilities that use ozone as the primary disinfectant, experiments were conducted on the effect of sequential exposure

of *C. parvum* to ozone followed by either free chlorine or monochloramine. Ransome et al. (1993) reported no synergy for ozone followed by free chlorine but used *in vitro* excystation to assess levels of inactivation.

1.2 Problem Statement and Research Objectives

Minimal information is available to quantify the resistance of *Cryptosporidium parvum* oocysts to inactivation by chemical disinfectants. The overall goal of this research was to examine the effect of various chemical disinfectants used in practice by the water treatment industry to control *C. parvum* in drinking water. Bench-scale experiments were conducted using batch reactors, laboratory water, and animal infectivity to measure changes in oocyst viability. Rather than use natural waters, laboratory water was used for all disinfection trials in order to minimize sources of experimental error due to batch variation.

Specific objectives of this research were to:

1. Identify a robust kinetic inactivation model able to describe the various types of microbial survival curves under disinfectant demand conditions (Chapter 2). Kinetic models are the fundamental basis for assessing the disinfection efficiency of chemical disinfectants and developing process design requirements for *C. parvum*.
2. Assess the effect of free chlorine at pH 6, preformed monochloramine at pH 8, and chloramination using post-ammoniation at pH 8 and 22°C on *C. parvum*. Kinetic inactivation models will be developed as experimental data permits (Chapter 3).

3. Investigate the kinetics of inactivation of *C. parvum* by ozone and design applications based on experimental data collected by Finch and co-workers (1993) in conjunction with cross-validation trials conducted in this work at 22°C (Chapter 4).

4. Assess the feasibility of using ozone followed by either free chlorine at pH 6 or monochloramine at pH 8 to control *C. parvum* at 22°C (Chapter 5).

1.3 Disinfection of Drinking Water

Water treatment facilities use multiple barriers, such as flocculation/sedimentation, filtration, and post-disinfection, to protect against outbreaks of waterborne disease. The purpose of disinfection in water treatment is to inactivate pathogenic microorganisms present in source waters and thereby prevent the transmission of waterborne disease. Microorganisms are classified as either viruses, bacteria, and protozoa and it is the waterborne pathogenic species within these groups that pose a threat to public health with regards to treatment of drinking water. Another concern is bacterial regrowth in water distribution systems causing biofilm growth and consequent taste, odor, and color problems, as well as enhanced corrosion of iron pipes by iron reducing bacteria.

1.3.1 Types of Microbes

Bacteria are single-celled prokaryotes which inhabit a wide variety of environments. Waterborne bacteria that are pathogenic to humans are usually transmitted through contamination of water supplies by human or animal feces, although *Vibrio cholerae* has been found in coastal waters uncontaminated by feces (Rheinheimer 1991). Bacteria producing the most significant health effects include *Salmonella* spp., *Shigella* spp., and

Vibrio spp. (Hoff 1986). Typhoid fever epidemics occasionally occur due to breakdown in water purification methods or contamination of water supplies by natural disasters or leaking sewer linings (Brock et al. 1984). Although *Bacillus* and *Clostridium* species are found primarily in the soil, they form highly resistant endospores which may be waterborne (Russell et al. 1994).

Viruses are obligate parasites because they cannot propagate outside of their natural host cell. Human enteric viruses tend to be stable in acid environments and at cold temperatures, though they are sensitive to heat, desiccation, and various oxidants (Foliguet et al. 1987). Waterborne viruses that are pathogenic to humans are extremely small, ranging in size from 21 to 30 nm for picornaviruses and 70 to 80 nm for adenoviruses and reoviruses (Brock et al. 1984). Removal of viruses by filtration systems at water treatment plants is challenging because of their small size. Adenoviruses, rotaviruses, reoviruses and enteric picarnoviruses are highly resilient and can remain viable for long periods of time in water (Cooper and MacCallum 1984). In general viruses are more resistant than bacteria to inactivation by disinfectants. Some waterborne viruses that cause significant health effects include Coxsackievirus A/B, Echovirus, Hepatitis type A, Norwalk type, and Poliovirus (Foliguet et al. 1987).

Protozoa are unicellular animals that, unlike bacteria and viruses, possess membrane-bound genetic material and other assorted cellular organelles. Protozoans are either free-living or parasitic and they are further categorized on the basis of their methods of locomotion and reproduction (Cox 1993). Parasitic protozoa typically inhabit the intestines of humans or animals in the form of trophozoites. The formation of

environmentally resistant cysts which pass out of the host in the feces allows the parasite to infect a new (suitable) host via ingestion of contaminated water (Wilson 1979). The information in Table 1-1 summarizes waterborne protozoans that are of significance to public health. *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium parvum* and *Balantidium coli* are only found outside of a host in the cyst form (Fayer and Ungar 1986). *Acanthamoeba* and *Naegleria* species may be found living freely in the environment in trophozoite as well as in cyst form (Cox 1993). Acanthamoebae typically infect chronically ill or immunosuppressed patients and like *Naegleria* species they are opportunistic pathogens entering the host through the olfactory mucous membrane (Piekarski 1989). No waterborne disease outbreak in North America has been associated with Acanthamoebae and *Naegleria* (AWWA 1995). Amongst protozoans, waterborne disease outbreaks are most often associated with *Entamoeba histolytica*, *Giardia*, and *Cryptosporidium parvum*.

G. lamblia, a human intestinal binucleate flagellated protozoan which causes prolonged diarrhea, is a leading cause of reported outbreaks of waterborne disease in the United States (Craun 1990; Kappus et al. 1994) and the most frequently isolated enteric protozoan from populations worldwide (Lengerich et al. 1994). Although giardiasis may be contracted through person-to-person contact, recreational activities, and food, infective cysts present in potable water are considered the primary source of *Giardia* epidemics (Foliguet et al. 1987; Roach et al. 1993).

1.3.2 *Cryptosporidium parvum*

Cryptosporidium parvum has been found in surface waters more frequently and in greater numbers than *G. lamblia* (Rose et al. 1988). *C. parvum* oocysts are considered to be present in 65 to 97% of surface waters in the United States (D'Antonio et al. 1985; Gallaher et al. 1989), and were reported to have breached water treatment filters in 27 to 54% of communities tested (Hayes et al. 1989). The resistance of *C. parvum* oocysts to inactivation by chemical disinfectants is greater than that of *Giardia* spp. cysts (Korich et al. 1990b; Smith et al. 1988). A number of municipal waterborne outbreaks of cryptosporidiosis (Soave and Armstrong 1986) lead to a greater awareness of the potential for waterborne transmission.

1.3.2.1 Biology

Cryptosporidium spp. are placed taxonomically within the phylum Apicomplexa, order Eucoccidiorida, suborder Eimeriorina, and family Cryptosporidiidae (Soave and Armstrong 1986). Morphological and cross-transmission studies suggest the existence of at least six different species of *Cryptosporidium*, these are (Webster 1993): *C. parvum* which can infect humans and other mammals; *C. muris* which is usually found in rodents; *C. baileyi* and *C. meleagridis* which can cause disease in birds; *C. serpentis* which is found in reptiles; and *C. nasorum* in fish. However, the only species that is an important cause of disease in humans is *C. parvum* (Current and Bick 1989; Smith and Rose 1990; Webster 1993). *Cryptosporidium* species infect epithelial surfaces, especially those along the gut, and can be found in a wide range of vertebrates, including humans. Cryptosporidiosis can be transmitted by means of person-to-person or animal-to-person

contact, ingestion of fecally contaminated water or food, or exposure to environmental surfaces contaminated with fecal material (Soave and Armstrong 1986). Humans can be infected by *C. parvum* at any age and only previous exposure to the parasite results in either full or partial immunity to challenge infections (Current and Bick 1989). The results of a study that used a *C. parvum* strain derived from calves suggested that the (median) infectious dose of oocysts for healthy human volunteers is 132 oocysts (DuPont et al. 1995).

The life cycle of *C. parvum* is illustrated in Figure 1-1 (Current and Garcia 1991). The sporulated oocyst which contains 4 sporozoites is the resistant stage found in the environment. The preferred site of infection is the ileum where sporozoites penetrate individual epithelial cells. Parasites reside on the luminal surface of the cells and are intracellular, enclosed by a thin layer of host cell cytoplasm. Multiple fission results in the formation of 8 merozoites within the meront. Free merozoites, released from Type I meronts, penetrate new cells and undergo merogony to form new meronts. Type I merozoites are thought to form a somewhat different meront (Type II) which contains only 4 merozoites. The Type II merozoite enters a sexual cycle, some enlarging to macrogametes while others undergo multiple fission inside cells to form 16 non-flagellated microgametes. Microgametes rupture from the microgametocyte and penetrate macrogametes to form a zygote. A resistant oocyst wall is then formed around the zygote, and following meiosis, 4 sporozoites are produced (sporogony). These oocysts are passed in the feces and into the environment. Approximately 20% of the oocysts produced in the gut fail to form a wall around the oocyst and only a series of membranes

surround the developing sporozoites. These oocysts are devoid of a wall and are referred to as "thin-walled oocysts." It is believed that the sporozoites produced from thin-walled oocysts can excyst while in the gut to infect new cells. Continuous recycling of Type I meronts and the release of sporozoites from ruptured thin-walled oocysts gives *C. parvum* two autoinfective cycles.

A number of issues remain unresolved and require further research, including: whether the number of oocysts usually present in drinking water is sufficient to cause illness in humans, if immunosuppressed persons are more susceptible to lower doses of oocysts than immunocompetent persons, and if the infectious dose for healthy humans varies amongst different strains of *C. parvum*.

1.3.2.2 Viability of Encysted Parasites

Although most ozone inactivation studies of *C. parvum* have used animal infectivity as the criterion of viability (Korich et al. 1990b; Langlais et al. 1990; Parker et al. 1993; Peeters et al. 1989), some studies have used *in vitro* excystation (Ransome et al. 1993; Sundermann et al. 1987). The viability of *Giardia* cysts has been determined by eosin exclusion (Bingham et al. 1979), fluorogenic dye staining (Jackson et al. 1985), determination of cyst morphology (Schupp and Erlandsen 1987a), *in vitro* excystation (Bingham and Meyer 1979; Schaefer 1990), and animal infectivity (Belosevic et al. 1983; Roberts-Thomson et al. 1976). Animal infectivity models provide information about the viability of cyst populations, ideal for disinfection studies, but no information regarding individual cysts. Nucleic acid stains could replace excystation as a rapid, inexpensive assay for viability of *Giardia* cysts in disinfection studies and could also provide the means

for defining the viability of cysts isolated from environmental samples (Taghi-Kilani et al. 1996).

For chemical disinfection studies, the U.S. EPA recommended the use of *in vitro* excystation which replaced eosin or vital dye-exclusion as the standard protocol for determining viability of *Giardia* cysts. Although excystation is an accepted method for assessing viability, it is subjective and likely to overestimate true cyst viability because counts include aborted attempts at trophozoite emergence from cysts (deRegnier et al. 1989; Schupp and Erlandsen 1987b). The use of excystation is preferred over that of animal infectivity because of the time and expense involved with animal models (Jarroll 1988), and its use is substantiated with the apparent correlation between *in vitro* excystation and the *G. muris*-murine system (Hoff et al. 1985).

Hoff et al. (1985) concluded that excystation is a reliable measure of the inactivation of *G. muris* cysts exposed to chlorine compared with animal infectivity based on a comparison of percent cysts killed. However, disinfection kinetic data are analyzed using the logarithm of survival ratios in order to normalize regression residuals and error variance (Haas 1988). Illustrated in Figure 1-2 is a comparison of *in vitro* excystation and animal infectivity inactivation ratios (log-units) for *C. parvum* oocysts and *Giardia* cysts exposed to either ozone or free available chlorine in bench-scale batch reactors. An examination of the data collected by Hoff and co-workers reveals that a 1.4 log-unit reduction in excystation of *Giardia* cysts corresponded to reductions in infectivity varying from 0.7 to 2.6 log-units. Based on data for *C. parvum*, a reduction in excystation of approximately 1.4 log-units corresponded to reductions in infectivity ranging from 2.3 to

4.6 log-units. Inactivation of protozoan cysts is typically underestimated by *in vitro* excystation, as shown in Figure 1-2, and its use as the criterion for viability in chemical disinfection studies can lead to disparate values for contact time and concentration compared with animal infectivity (deRegnier et al. 1989). All of the ozone inactivation data used in this study are based on animal infectivity which is generally regarded as the best method for determining protozoan survival ratios in disinfection studies (deRegnier et al. 1989; Labatiuk et al. 1991).

1.3.3 Disinfectants

Disinfection of drinking water is practiced using chemical agents including chlorine compounds, bromine, iodine, and ozone, or energy-related processes such as ultraviolet (UV) and gamma radiation. Inactivation of microorganisms follows three major modes of action: destruction or impairment of semi-permeable cellular membranes or nucleic acids; interference with energy-yielding metabolism, for example prevention of oxidative phosphorylation; and interference with the biosynthesis of proteins, nucleic acids, and coenzymes, thereby inhibiting cell growth (James M. Montgomery Consulting Engineers 1985). It is has been postulated that the effectiveness of a disinfectant is influenced by two primary mechanisms: oxidation of the cell (or cyst) wall resulting in release of cellular constituents; and diffusion into the cell (or cyst) resulting in disruption of normal cellular activity (James M. Montgomery Consulting Engineers 1985). The efficacy of a chemical disinfectant is thus dependent on both it's ability to oxidize biological molecules and to diffuse across cell or cyst walls.

Standard oxidation-reduction potentials, a measure of a disinfectant's oxidizing strength, are given in Table 1-2 for chemical disinfectants used by the water treatment industry. Diffusion across the cell or cyst wall is dependent on a number of factors including molecular weight and charge. Amongst the halogens, the diffusion order is iodine > bromine > chlorine which is the reverse of oxidation potential ranking (James M. Montgomery Consulting Engineers 1985).

1.3.3.1 Chlorine

Chlorine is widely used in water treatment both as a disinfectant and as an oxidizing agent for taste and odour control, color removal, oxidation of iron(II) and manganese(II), sulfide oxidation, and ammonia removal (Snoeyink and Jenkins 1980). Chlorine is most often applied as a gas, $Cl_{2(g)}$, which rapidly dissolves in water to form molecular chlorine, $Cl_{2(aq)}$, which hydrolyzes to hypochlorous acid (HOCl), chloride ions (Cl^-), and hydrogen ion (H^+).

$$Cl_{2(aq)} + H_2O \Leftrightarrow HOCl + Cl^- + H^+$$

In dilute solution and at pH >4, this reaction achieves equilibrium within seconds leaving negligible $Cl_{2(aq)}$ (Snoeyink and Jenkins 1980). The addition of chlorine as a gas will lower the alkalinity of a water because of the production of a strong acid (HCl). Chlorine can also be applied as a salt of hypochlorous acid such as sodium hypochlorite:

$$NaOCl \rightarrow Na^+ + OCl^-$$

$$OCl^- + H_2O \Leftrightarrow HOCl + OH^-$$

Both methods for applying chlorine produces HOCl which is a weak acid that dissociates to H^+ and hypochlorite ion (OCl^-):

$$HOCl \Leftrightarrow H^+ + OCl^-$$

according to a dissociation constant (K_a) of 2.6×10^{-8} moles/L at 20°C (Morris 1966). At 20°C and pH 6.0, 7.0, and 8.0, HOCl constitutes 97.5, 79.3, and 27.7%, respectively, of the free chlorine, the quantity of HOCl, OCl^-, and any trace levels of $Cl_{2(aq)}$ (White 1992). Dissociation of HOCl is also dependent on temperature. Morris (1966) developed a formula to relate the acid dissociation constant to temperature:

$$pK_a = \frac{3000}{T} - 10.0686 + 0.0253T$$

where T is the temperature in °K. This relationship indicates that for a given pH, the fraction of HOCl will be lower at higher temperatures.

Chlorine is a strong oxidizing agent and will react with inorganic reducing agents such as reduced forms of iron (II) and sulfur compounds, organic compounds such as fulvic and humic acids, and ammonia present in water. The reaction of chlorine with humic material produces chlorinated organics, trihalomethanes (THMs), thought to be potential carcinogens (White 1992). Chlorine (HOCl) and ammonia (NH_3) react to form products known as (inorganic) chloramines which consist of three species: monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and trichloramine (NCl_3) (Haas 1990).

$$NH_{3(aq)} + HOCl \Leftrightarrow NH_2Cl + H_2O$$
$$NH_2Cl + HOCl \Leftrightarrow NHCl_2 + H_2O$$
$$NHCl_2 + HOCl \Leftrightarrow NCl_3 + H_2O$$

Each of these inorganic chloramines contributes to the total combined chlorine residual. Total available chlorine residual refers to the sum of the free available chlorine (FAC) and reactive (inorganic and organic) chloramines. The amount of chlorine that must be added to water before a stable free chlorine residual can be produced is called the "breakpoint." Under ideal conditions the breakpoint occurs at a chlorine to ammonia (as nitrogen) weight ratio of 10:1 (Haas 1990). The species of chloramine formed depends on a number of factors including the chlorine to ammonia (as nitrogen) weight ratio, pH and temperature. For chlorine to ammonia weight ratios less than 5:1, the predominant species formed is monochloramine at pH >7 (Snoeyink and Jenkins 1980). At lower pH values or higher chlorine to ammonia weight ratios, dichloramine may also be formed.

1.3.3.2 Ozone

Ozone (O_3), an allotrope of oxygen, is a highly reactive gas produced by passing extra dry air or oxygen through a "corona discharge". Ozone produced using UV and electrolysis of aqueous solutions of certain electrolytes generates low concentrations of ozone insufficient for water treatment facilities (Rice and Bollyky 1982). The maximum practical solubility of ozone in water is about 40 mg/L (Haas 1990). Ozone reactions in water are categorized as either direct oxidation, which is relatively slow and highly selective, or autodecomposition involving the hydroxyl radical ($OH^{\bullet}$) (Hoigné and Bader 1975). Experimental half-lives of ozone are 10^7 greater at pH 1 than at pH 13 (Munter 1985). In addition to pH, other factors influencing ozone decomposition in water include temperature, inorganic and organic compounds, and the presence of initiator compounds (Hoigné and Bader 1979; Roth and Sullivan 1983).

Chemical compounds involved in chain reactions are classified as initiators, promoters, or terminators (Staehelin and Hoigné 1985). Initiators (in either pure or natural water) such as hydroxyl ions react with ozone to form by-products which either directly or indirectly continue to react with ozone. Other examples of initiators include hydrogen peroxide, formate, humics and ferric ions. Promoters such as formic acid and humics react with ozonation by-products such as $OH^{\bullet}$ forming other by-products which in turn react with ozone. Chain terminators, or radical scavengers, such as carbonate and bicarbonate ions can reduce the rate of ozone decomposition by reacting with ozonation by-products like $OH^{\bullet}$ to form relatively inert products (Hoigné 1982; Tomiyasu et al. 1985). Low pH conditions favor the slow direct oxidation reactions involving ozone and high pH conditions favour autodecomposition.

The inactivation of microorganisms exposed to ozone has been attributed to the direct ozone reaction pathway based on studies of the effect of pH on disinfection efficiency (Ross et al. 1976; Venosa 1972). An advanced oxidation study observed less inactivation at higher ratios of peroxide to ozone that give lower ozone residuals (Wolfe et al. 1989). Although $OH^{\bullet}$ radicals are among the most reactive oxidants in water, radical species are thought to be less effective disinfectants (not oxidants) than molecular ozone because they are immediately consumed upon formation and display little substrate selectivity (Hoigné 1982).

1.3.4 Water Quality Characteristics

Disinfection efficiency is influenced by water quality characteristics such as turbidity, organics, pH and temperature. Particulates responsible for turbidity can shield

microorganisms from disinfectants and interfere with the disinfection process (James M. Montgomery Consulting Engineers 1985). Organics can adhere to cell surfaces and react with the disinfectant to form compounds with minimal or no effect on microbial survival. The pH of a water may affect the speciation of a chemical disinfectant, or promote rapid disappearance of an ozone residual, thereby influencing disinfection efficiency. The rate of diffusion of the disinfectant through the cell or cyst wall and the rate of chemical reaction with the organism is influenced by temperature.

1.3.5 Indicator Organisms

Many waterborne pathogens, including *C. parvum*, are difficult to detect in water. Instead, drinking water is checked for indicator organisms whose presence indicates fecal contamination (Brock et al. 1984). In this regard, an ideal indicator organism would possess the following characteristics (James M. Montgomery Consulting Engineers 1985):

- abundant in feces and sewage;
- largely absent from other sources, including the natural environment;
- nonpathogenic;
- more resistant to disinfection and environmental stress than pathogens;
- more numerous than pathogens;
- easily isolated, identified, and enumerated.

Coliform bacteria are the most widely used indicator organisms. This group includes all the aerobic and facultatively anaerobic, gram-negative, non-spore forming, rod-shaped bacteria that ferment lactose with gas formation within 48 h at 35°C (Greenberg et al. 1992).

Alternative indicator organisms of interest in the field of water treatment include coliphages, *Mycobacterium*, and heterotrophic plate count (HPC) bacteria (Payment and Franco 1993; Rheinheimer 1991). Coliphages, viruses which use coliform bacteria as host cells, have been suggested as indicators of enteric viruses in water (Grabow and Coubrough 1986). Mycobacteria are likely the most resistant of all non-spore forming bacteria to chemical agents including ozone (Engelbrecht et al. 1979); however, some are pathogenic such as *M. tuberculosis* (Langlais et al. 1991). Heterotrophic bacteria grow in water distribution systems provided that environmental conditions such as temperature, water velocity, and residence time are favorable (LeChevallier et al. 1987). HPC bacteria counts >500 CFU/mL have been found in water distribution systems unable to maintain chloramine residuals of 0.2 mg/L (LeChevallier et al. 1990).

1.3.6 Regulatory Issues

Relatively high rates of waterborne disease outbreaks have been associated with water treatment facilities only providing disinfection of surface waters and neither disinfection nor filtration of groundwaters (Craun 1988). All surface waters are at risk for protozoan contamination, as well as groundwaters which are in direct contact with surface waters. A multiple barrier approach is required to prevent further outbreaks of waterborne disease.

To safeguard against waterborne outbreaks of giardiasis, the U.S. EPA updated the Surface Water Treatment Rule (SWTR) in 1990 to require 3 log-units removal-inactivation of *Giardia* cysts and 4 log-units removal-inactivation of viruses from surface waters (USEPA 1989). This disinfection requirement applies regardless of the quality of

the source water. Water utilities must provide disinfection of surface and ground waters, in addition to filtration. Utilities may be exempted from providing filtration if chemical disinfection is adequate for 3 log-units inactivation of *Giardia* cysts (and 4 log-units inactivation of enteric viruses) if all of the following conditions are satisfied:

- fecal and total coliforms are <20 and <100 CFU/mL, respectively, in 90% of raw water samples;
- raw water turbidity is <5 NTU;
- the distribution system has a disinfectant residual >0.20 mg/L (to ensure HPC bacterial counts <500 CFU/mL) and meets the Total Coliform Rule and Trihalomethane (THM) requirements;
- an effective watershed control program is in place;
- an annual on-site inspection is conducted; and
- the utility can validate the absence of waterborne disease occurrences.

The Total Coliform Rule suggests that the number of total coliforms be <1 CFU/100 mL. A disinfectant residual greater than 0.2 mg/L in the distribution system should ensure heterotrophic bacteria densities <500 CFU/mL. The maximum THM concentration of water must be less than 100 µg/L on annual average. The Total Coliform Rule regulates the coliform group of microorganisms (total, fecal, *E. coli*) which have traditionally been used as indicators of pathogen contamination. However, coliform organisms are inadequate for predicting the possible presence of *C. parvum* oocysts and other protozoans in treated drinking water because of their relatively higher resistance to chemical disinfectants such as chlorine.

Inactivation-removal requirements for *C. parvum* are likely to be included in the Enhanced Surface Water Treatment Rule (ESWTR). Water quality data collected in the U.S. under the Information Collection Rule (ICR) will be used to establish the required performance standards. Occurrence data for *C. parvum* in source waters is to be used for setting required levels of oocyst removal-inactivation. Utilities are likely to detect oocysts in their surface source water occasionally, and it is thought that 24 to 50% of utilities may detect oocysts in treated waters (Sorvillo et al. 1992). The performance criterion for *C. parvum* may range from 2.0 to 6.0 log-units removal-inactivation.

Canadian water utilities must satisfy "The Guidelines for Canadian Drinking Water Quality" (Health and Welfare Canada 1993) which regulates maximum contaminant levels of certain chemicals and microorganisms. Regulatory issues related to water disinfection include acceptable levels of: turbidity, microorganisms, and disinfection by-products. The maximum turbidity entering the distribution system must be ≤1 NTU (when it is demonstrated that disinfection is not compromised, turbidity may increase to ≤5 NTU). At this time there are no regulations regarding protozoans or viruses. The maximum concentration of THMs permitted in drinking water is currently 350 μg/L.

1.4 References

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Table 1-1. Pathogenic waterborne protozoa

Protozoan	Host environment(s)	Disease	Size
Acanthamoeba spp.	Fresh water, damp soil	Granulomatous amoebic encephalitis	trophozoite: 15-45 μm cyst: 15-20 μm
Balantidium coli	Pigs, humans	Balantidial dysentery	trophozoite: 50-150 × 50-170 μm cyst: 50 μm
Cryptosporidium spp.	Animals, humans	Cryptosporidiosis	sporozoite: 5-6 μm oocyst: 3-6 μm
Entamoeba histolytica	Humans	Amoebic dysentery	magna form: 20-30 μm minuta form: 10-20 μm cyst: 10-15 μm
Giardia lamblia	Animals, humans	Giardiasis (gastroenteritis)	trophozoite: 12-15 × 6-8 μm cyst: 7-12 μm
Naegleria spp.	Fresh water, damp soil	Primary amoebic meningoencephalitis	trophozoite: 22 × 7 μm cyst: 9 μm

Source: (Cox 1993; Piekarski 1989)

Table 1-2. Oxidation-reduction potentials of chemical disinfectants at 25°C

Compound	Half Reaction	Potential, Volts
Ozone	$O_{3(g)} + 2H^+ + 2e^- \Leftrightarrow O_2 + H_2O$	+2.07
Hypochlorous acid	$HOCl + H^+ + 2e^- \Leftrightarrow Cl^- + H_2O$	+1.49
Molecular chlorine	$Cl_2 + 2e^- \Leftrightarrow 2Cl^-$	+1.36
Chlorine dioxide	$ClO_2 + e^- \Leftrightarrow ClO_2^-$	+1.15
Bromine	$Br_2 + 2e^- \Leftrightarrow 2Br^-$	+1.07
Hypochlorite ion	$OCl^- + H_2O + 2e^- \Leftrightarrow Cl^- + 2OH^-$	+0.90
Iodine	$I_{2(aq)} + 2e^- \Leftrightarrow 2I^-$	+0.54

Source: (Degrémont 1979; White 1992)

Source of Copyrighted image: Current, W. L., and Garcia, L. S. (1991). "Cryptosporidiosis." Clinical Microbiology Reviews, 4, 325-358.

Figure 1-1. Life cycle of *Cryptosporidium parvum*

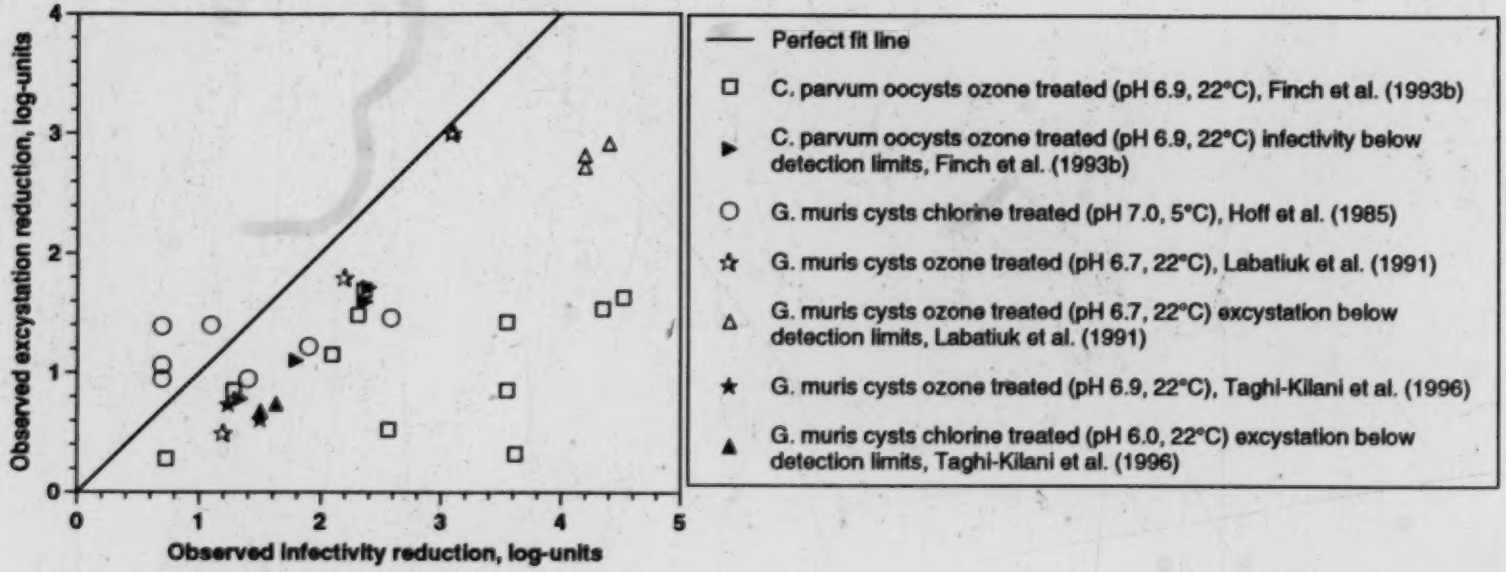
Observed excystation reduction, log-units
Observed infectivity reduction, log-units
0
1
2
3
4
5
Perfect fit line
C. parvum oocysts ozone treated (pH 6.9, 22°C), Finch et al. (1993b)
C. parvum oocysts ozone treated (pH 6.9, 22°C) infectivity below detection limits, Finch et al. (1993b)
G. muris cysts chlorine treated (pH 7.0, 5°C), Hoff et al. (1985)
G. muris cysts ozone treated (pH 6.7, 22°C), Labatiuk et al. (1991)
G. muris cysts ozone treated (pH 6.7, 22°C) excystation below detection limits, Labatiuk et al. (1991)
G. muris cysts ozone treated (pH 6.9, 22°C), Taghi-Kilani et al. (1996)
G. muris cysts chlorine treated (pH 6.0, 22°C) excystation below detection limits, Taghi-Kilani et al. (1996)

Figure 1-2. Comparison of excystation and animal infectivity measures of *C. parvum* oocysts and viability of *G. muris* cysts following exposure to ozone and free chlorine in phosphate buffered laboratory water

Chapter 2

Water Treatment Disinfection Kinetics[1]

2.1 Introduction

The purpose of potable water disinfection is the inactivation of pathogenic microorganisms to prevent transmission of waterborne disease. The most frequently used disinfectant is chlorine. Historically, chlorine disinfection in water treatment was considered satisfactory if no coliform bacteria were detected. A typical disinfection system was designed to provide efficient mixing of chlorine solution with raw water for a contact time of at least 30 min with the concentration of free chlorine in the reactor effluent between 0.1 and 0.2 mg/L (James M. Montgomery Consulting Engineers 1985). The detection of organisms that are relatively resistant to free (available) chlorine, such as protozoa, in addition to health concerns regarding chlorination by-products, has lead to increased use of alternative chemical disinfectants such as chlorine dioxide and ozone (Hubbs et al. 1980; Sobsey 1989; Suh and Abdel-Rahman 1985). Because it is not currently possible to set an implementable maximum contaminant level for enteric viruses and *Giardia* cysts, treatment requirements based on characteristic disinfectant concentration × characteristic contact time (CT) products were promulgated (USEPA 1989) and intended to provide adequate control of these organisms under the U.S. Surface Water Treatment Rule (SWTR). At present the SWTR requires 3 and 4 log-units removal-inactivation of *Giardia* sp. cysts and viruses, respectively. The SWTR credits filtration at conventional treatment plants with 2.5 and 2 log-units removal for *Giardia*

[1] Gyürék, L.L. and G.R. Finch paper submitted to *Journal of Environmental Engineering*, (August, 1997).

and viruses, respectively (USEPA 1990). The SWTR Guidance Manual specifies levels of inactivation credited based on calculated CT values for several disinfectants. The Guidelines for Canadian Drinking Water Quality do not provide minimum disinfection requirements for protozoa and viruses (Health and Welfare Canada 1993).

Effective control of resistant pathogens in potable water not only requires the use of effective disinfectants but also requires the need for optimal design criteria to ensure protection of public health and to minimize risk from long term exposure to disinfection by-products. Microbial inactivation rate laws and kinetic models are the basis for assessing disinfection performance and the design of contactor systems. Over the years a number of kinetic models have been proposed for the development of disinfection design criteria. A second issue, in addition to model adequacy, is that of chemical disinfectant disappearance during the contact time. Most kinetic models have been derived for disinfectant demand-free conditions where the concentration is (approximately) constant during the contact time. Kinetic models can be applied to disinfectant demand conditions (Lawler and Singer 1993). Accounting for the disinfectant concentration profile during the contact time improves the disinfection design criteria provided that the kinetic model adequately describes inactivation behavior.

This paper serves as a compendium of kinetic models used in water treatment for both disinfectant demand-free and demand conditions. Data describing ozone inactivation of heterotrophic plate count (HPC) bacteria are used to assess the adequacy of the various kinetic models. One outcome of this review will be the recommendation of a robust model for the design of contactor systems to ensure that treatment requirements are

satisfied. This discussion is limited to batch and flow through reactors with perfect plug flow characteristics.

2.2 Inactivation kinetics under disinfectant demand-free conditions

Various types of microbial inactivation curves are illustrated in Figure 2-1 which is a semi-log plot of inactivation with contact time (Berg et al. 1988; Severin and Churn 1987) Curve A represents exponential death or first-order inactivation kinetics. Curve B displays exponential kinetics following an initial shoulder or lag, that has been attributed to inadequate mixing, delays in diffusion of the disinfectant to sites of action, and/or multiple targets necessary for inactivation (Hoff 1987). Multiphasic curves C and D are attributed to clumping of organisms and the presence of many distinct subpopulations with varying resistance to a disinfectant or a distributed inactivation resistance within one population (Hiatt 1964; Cerf 1977; Gold 1977; Engelbrecht et al. 1980). Curve D illustrates tailing-off behavior in which rapid initial inactivation is followed by a decrease in the activation rate yielding a plateau. The resistant fraction of a microbial population exposed to a chemical disinfectant may be significantly greater in populations living in nutrient-limited conditions compared with organisms grown under optimum conditions, such as those typically employed in laboratory disinfection studies (Carson et al. 1972; Harakeh et al. 1985). Bacteria grown in chemostats at sub-maximum rates are more resistant to chlorine dioxide than bacteria that are batch-grown at the optimum growth rate (Berg et al. 1988). This disparity has been attributed to growth phase differences at the time of disinfectant exposure and physiological dissimilarities in the bacterial cells (Berg et al. 1982). Kinetic

inactivation models have been advanced to describe mathematically the curves A to D in Figure 2-1.

2.2.1 Kinetic models

An essential feature of kinetic modeling is simplification and idealization of complex phenomena. Models attempt to represent interactions of chemicals with different cellular targets and modes of action with highly complex microorganisms. Kinetic inactivation models are derived based on the following assumptions being satisfied for batch reactors (Severin and Churn 1987): no back mixing; uniform dispersion of organisms and disinfectant molecules; sufficient mixing to ensure that liquid diffusion is not rate limiting; temperature and pH are fixed; and the disinfectant concentration is assumed to remain constant during the contact time. The stability of chlorine species permits the addition of chlorine as an aliquot to reactors in which washed preparations of organisms are suspended in oxidant demand-free solution. Whereas chlorine dioxide and ozone are less stable and disappear during the contact time. To maintain a constant ozone residual, semi-batch experimental protocols have been used in which an oxygen-ozone gas mixture is continuously supplied to a batch reactor (Wickramanayake and Sproul 1988). Several kinetic models can be derived from the following differential rate law:

$$\frac{dN}{dt} = -kmN^{x}C^{n}t^{m-1} \qquad (2\text{-}1)$$

where dN/dt is the rate of microbial inactivation; N is the number of survivors at contact time t; k is a reaction rate constant found experimentally; C is the concentration of the disinfectant; and m, n, and x are empirical constants. Kinetic models are summarized in Table 2-1.

2.2.1.1 Chick-Watson model

Chick (1908) studied disinfection reaction rates by exposing bacteria to a constant disinfectant concentration in test tubes and enumerating survivors at successive time intervals. The reaction between the protoplasm of bacteria and the chemical disinfectant was considered by Chick to be analogous to an elementary bimolecular chemical reaction:

$$xN + nC \xrightarrow{k_1} p(\text{inactive microbe})$$

This reaction is described by the following rate law:

$$\frac{dN}{dt} = -k_1 N^x C^n \cdot (\text{inactive microbe})^p$$

where x and n equal unity. Assuming that the disinfectant is present in excess and that the reaction is irreversible, i.e. p=0, the resulting expression is known as Chick's law:

$$\frac{dN}{dt} = -k^* N^1$$

where k^* is a pseudo first-order reaction rate constant equal to $k_1 C$. Chick's rate law states that the number of bacteria destroyed per unit time is proportional to the number remaining for a given concentration of disinfectant. Integration of Chick's law gives the pseudo-first order relationship:

$$\ln \frac{N}{N_o} = -k^* T$$

where T is the applied contact time, min. Of Chick's original work only anthrax spores disinfected with phenol conformed to this first-order relationship. Watson (1908) proposed an empirical logarithmic function to account for the effect of different (fixed) disinfectant concentrations:

$$k = C^n T$$

where k is a constant for a specific microorganism and set of conditions; n is a constant referred to as the coefficient of dilution which represents the average number of molecules that have combined with the organism to cause inactivation; and T is the time required to achieve a given level of inactivation. Values of n less than unity indicate contact time that is more important than disinfectant dose for a specified level of kill.

Chick's pseudo first-order reaction rate constant (k^*) can be expressed by means of Watson's function using (Langlais et al. 1991):

$$k_1 C^1 = k^* = kC^n$$

Incorporation of Watson's function into Chick's rate law gives the Chick-Watson pseudo first-order rate law:

$$\frac{dN}{dt} = -kNC^n \qquad (2\text{-}2)$$

where k is a first-order rate constant. For x=1 (and m=1), the generalized differential rate law simplifies to equation 2-2 which can be integrated to give the Chick-Watson model:

$$\int_{N_o}^{N} \frac{dN}{N} = -kC^n \int_0^T dt$$

$$\ln \frac{N}{N_o} = -kC^n T$$

$$\log \frac{N}{N_o} = -kC^n T \qquad (2\text{-}3)$$

where : $k = \dfrac{k}{\ln(10)}$

The simplicity of the Chick-Watson model makes it attractive for design and regulatory practice. In general, disinfection systems are designed using CT products derived from Chick-Watson kinetics based on laboratory inactivation studies (Hoff 1987). The Safe Drinking Water Committee (1980) chose CT values, typically for a 99% level of inactivation, as the method for comparing biocidal efficacy for a given microorganism and set of conditions. The Chick-Watson model cannot describe deviations from first-order inactivation kinetics represented by curves B, C, and D in Figure 2-1.

Chlorine inactivation of homogenous bacterial populations has been shown to follow exponential death (Haas and Karra 1984a). Ozone inactivation of *Escherichia coli* (ATCC 11229) was adequately described by Chick-Watson kinetics (Zhou and Smith 1994). However, bacteria were grown under ideal laboratory conditions and a complete mix flow reactor (CMFR) was used rather than a batch reactor. A CMFR set-up typically consists of two reservoirs and a reactor. Stock oxidant solution and a microbial suspension are simultaneously and continuously fed to the reactor and allowed to achieve steady-state conditions (Selleck et al. 1978). It is common to use a CMFR when the rate of (bacterial) inactivation is extremely fast such as with ozone (Severin and Churn 1987). A CMFR slows the reaction so as to minimize sampling errors; unfortunately, it also masks the true inactivation behavior. By design, a fraction of the population will exit the CMFR having received less than the average disinfectant dose (Severin and Churn 1987). For organisms exhibiting a tailing-off effect in batch systems, survival ratios in CMFRs will be less than otherwise expected. Severin and Churn (1987) found that the Chick-Watson model adequately describes ultraviolet radiation inactivation kinetics of *E. coli*

and *Candida parapsilosis* suspended in CMFRs, even though it was evident from batch data that these organisms did not adhere to Chick-Watson kinetics. For a given level of inactivation, the CT value for a CMFR can be orders of magnitude greater than the CT value for a batch or plug-flow reactor (Severin and Churn 1987), presumably because of hydraulic short circuiting in the CMFR. Studies involving numerous chemical disinfectants and organisms representative of naturally occurring populations have shown significant deviations between observed kill and that predicted by Chick-Watson kinetics in batch studies (Fair et al. 1948; Hiatt 1964; Hom 1972; Hoff 1986; Anmangandla 1993; Finch et al. 1993b). The use of CT values can lead to over or under design of disinfection systems.

According to Langlais et al. (1991) the Watson function is based on the assumption that microorganisms are genetically similar, the same strain, and that the killing action is a single-hit and single-site type. These assumptions are necessary to derive the Chick-Watson model based on a chemical reaction mechanism. Modeling microbial inactivation behavior as that of reagent grade chemicals reacting with chemical disinfectants in an irreversible, bimolecular, elementary reaction is overly simplistic (Hoff 1987). Microorganisms are complex structures and many different transport mechanisms and chemical reactions must occur to inactivate one organism (Venkobachar et al. 1977; Jacangelo et al. 1991).

2.2.1.2 Hom model

Observing that plots of log survival on contact time for chlorine disinfection of natural algal-bacterial systems were curvilinear rather than linear, Hom (1972) made a useful empirical generalization of the Chick-Watson pseudo first-order rate law:

$$\frac{dN}{dt} = -kmNC^n t^{m-1} \tag{2-4}$$

Integration of this rate law gives the semi-empirical Hom model:

$$\int_{N_o}^{N} \frac{dN}{N} = -kmC^n \int_0^T t^{m-1} dt$$

$$\log \frac{N}{N_o} = -kC^n T^m \tag{2-5}$$

Fair et al. (1948) similarly observed disinfection data to be curvilinear and found that log survival when plotted on t^2 gave a straight line, implying a value of m=2 in the Hom model. A formal model was not proposed based on this observation. This finding is consistent with a mechanistic model when derived assuming the existence of an intermediate disinfectant-organism complex such as described for Poliovirus type I (Mahoney) by hypochlorous acid (Haas 1980).

The level of inactivation predicted by the Hom model is a nonlinear function of C and T, dependent on the model parameters n and m, respectively. The model can describe curves A to D illustrated in Figure 2-1 and simplifies to the Chick-Watson model for n=1 and m=1. For the case in which m is greater than unity, the survival curve displays an initial shoulder; a tailing-off effect is produced when m is less than unity (Haas and Karra 1984a).

2.2.1.3 Rational model

A power law kinetic expression has been used to describe viral inactivation in systems with a constant ozone residual (Majumdar et al. 1973; Roy et al. 1981):

$$\frac{dN}{dt} = -kN^x C^n \tag{2-6}$$

which is the generalized rate law for m=1. Integration of this rate law gives the Rational model:

$$\int_{N_o}^{N} \frac{dN}{N^x} = -kC^n \int_0^T dt$$

where $x \neq 1$

$$N^{1-x} - N_o^{1-x} = (x-1) \cdot kC^n T$$

$$\left[\frac{N}{N_o}\right]^{1-x} = 1 + (x-1) \cdot kC^n T \cdot N_o^{x-1}$$

$$\log \frac{N}{N_o} = -\frac{\log\left[1 + N_o^{x-1}(x-1)kC^n T\right]}{(x-1)} \tag{2-7}$$

The Rational model can describe shoulder and tailing-off phenomena for x less than or greater than unity, respectively. Roy et al. (1981) used a CMFR experimental set-up to study ozone inactivation of Poliovirus type 1 and estimated x to be 0.69.

2.2.1.4 Hom-Power law model

The generalized differential rate law, equation 2-1, can be integrated as previously shown for the Rational model but with parameters m and x both present to give the Hom-Power law model (Anotai 1996):

$$\log\frac{N}{N_o} = -\frac{\log\left[1+N_o^{x-1}(x-1)kC^nT^m\right]}{(x-1)} \qquad (2\text{-}8)$$

This model may provide a somewhat better fit than either the Hom or Rational models for survival curves in which the inactivation rate initially increases and then decreases during the contact time, such as curve C in Figure 2-1. A four parameter kinetic model may lead to overparametrization resulting in highly correlated parameter estimates.

2.2.1.5 Selleck model

The following rate law was proposed by Gard (1957) to describe an observed decrease in the rate of poliovirus inactivation during the contact time even when the concentration of formaldehyde remained constant:

$$\frac{dS}{dt} = -\frac{kCS}{1+kCT}$$

where S is the microbial survival ratio N/N_0 at contact time t; k and k are rate constants; C is the disinfectant concentration, mg/L; and T is the total applied contact time, min. A gradual decrease in permeability caused by the reaction of the disinfectant with the viral protein coat was postulated as a mechanistic rationale for this kinetic behavior (Hiatt 1964). This rate law was later modified to describe chlorine inactivation of coliform bacteria in wastewater effluent (Selleck et al. 1978):

$$\frac{dS}{dt} = \frac{nC}{k}S^{(n+1)/n} \qquad (2\text{-}9)$$

where n and k are constants. Integration of this expression gives the Selleck model (Haas 1979):

$$\int_{1}^{S} \frac{dS}{S^{(n+1)/n}} = -\frac{nC}{k}\int_{0}^{T} dt$$

$$-n(S^{-1/n}+1) = -n\frac{CT}{k}$$

$$S = \left[1+\frac{CT}{k}\right]^{-n}$$

$$\log\frac{N}{N_o} = -n\log\left[1+\frac{CT}{k}\right] \qquad (2\text{-}10)$$

This empirical model has been used to describe survival curves having an initial shoulder and a declining inactivation rate which are often observed in chlorination of wastewater (curve C in Figure 2-1) with values of k and n related to the chlorine to nitrogen dose ratio (Haas and Karra 1984b).

2.2.1.6 Multiple-target

Multiple-target theory postulates that each organism or clump of organisms possesses a total of n_c identical, independent, critical targets all of which must be hit once in order to destroy the particle (Hiatt 1964). The term particle is used because it is impossible to identify the difference in the survival of a clump of organisms or a single organism (Severin et al. 1983). Target theory originated from irradiation studies on microorganisms (Harm 1980). The concept was then used to explain initial shoulders in survival curves of microorganisms exposed to a constant chemical concentration (or ultraviolet radiation intensity). Assuming that mixed second-order kinetics describe the rate of inactivation of a critical target the probability of inactivating a target is given by:

$$P_{1-t} = 1 - e^{-kCT}$$

where k is the inactivation rate constant, L/(mg·s); C is the chemical disinfectant concentration, mg/L; and T is the contact time (Severin et al. 1983). The probability of survival of a particle with n_c critical targets is given by (Kimball 1953):

$$\log \frac{N}{N_o} = \log(P_t) = \log\left[1 - (1 - e^{-kCT})^{n_c}\right] \qquad (2\text{-}11)$$

This statistical derivation of the Multiple-target model assumes that all targets are equal in all aspects. The Multiple-target model is not suitable for describing microbial inactivation kinetics because it is unlikely that clumps will be of equal size, and that damage is randomly distributed among targets (Wei and Chang 1975).

2.2.1.7 Series-event (Poisson) models

Series-event models have been proposed to account for shoulders observed on survival curves and the cumulative effect of chemical disinfectants on microbial targets during the contact time. The inactivation of a single organism can be idealized as occurring in a series of damaging reactions which occur in integer steps (Severin et al. 1984). The rate at which an organism passes from one event level to the next is considered first-order with respect to chemical concentration. Unlike the Multiple-target model, the Series-event model assumes that only a finite number of lethal events, *l*, from an infinite number of targets need to occur to inactivate an organism. Organisms which accumulate less than the postulated number of lethal reactions, *l*, survive the disinfection process. The number of potential reaction sites greatly exceeds the lethal number, *l*, such that the rate at which an organism attains lethal damage is not affected by the depletion of

available sites. These random collisions between oxidant molecules and microorganisms can be expressed as a Poisson probability because the number of collisions greatly exceeds the number of organism deaths (Wei and Chang 1975). Assuming that the destruction of the l^{th} target occurs at the same rate as the destruction of the first target, the rate of destruction of a κ^{th} site in an organism is given by:

$$\frac{dN_\kappa}{dt} = kCN_{\kappa-1} - kCN_\kappa$$

Solving for $\kappa=0$ to $\kappa=l-1$ gives the log fraction of organisms surviving, those not exceeding $l-1$ at the end of the contact time:

$$\log\frac{N}{N_o} = \frac{-kCT + \ln\left[\sum_{\kappa=0}^{l-1}\frac{(kCT)^\kappa}{\kappa!}\right]}{\ln(10)} \qquad (2\text{-}12)$$

Considering the Series-event model to be conceptually the same for single organisms or uniform clumps of organisms, Wei and Chang (1975) used a similar model to describe chlorine inactivation of clumps, the number of organisms per clump varying from 1 to 18. However, this is inappropriate because the Series-event model assumes the occurrence of an event in a viable organism is independent of the occurrence of previous events (Severin et al. 1983). When an event occurs in a clump, it is possible that this event occurs in an organism which is already past the threshold level l thereby making the event irrelevant for inactivating the clump. The probability of entirely inactivating a clump of organisms is given by:

$$P_{1-t} = \left[1 - \frac{N}{N_o}\right]^L$$

where L is the number of organisms per clump. The probability of survival of a clump can thus be expressed as the Multiple series-event model:

$$\frac{N_c}{N_{c_o}} = 1 - \left(1 - e^{-kCT} \sum_{\kappa=0}^{l-1} \frac{(kCT)^{\kappa}}{\kappa!}\right)^{L} \qquad (2\text{-}13)$$

The Multiple series-event model simplifies to the Multiple-target model when uniform clumps are assumed to be made of single-hit organisms, given by l=1, and hence the number of organisms per clump, L, equals the number of critical targets, n_c. When there is no clumping, L=1, and all organisms are of the single-hit type, l=1, this model simplifies to the Chick-Watson model (equation 2-3) for n=1. The Multiple series-event model has not been shown to better describe typical inactivation data compared with Series-event or Multiple-target models (Severin et al. 1984).

The Chick-Watson rate law (equation 2-2) assumes that inactivation of an organism occurs in a single n^{th} order reaction with respect to chemical concentration. The Series-event model assumes a number of reactions, l lethal events, which are first-order with respect to concentration are needed to inactivate the organism. This latter assumption is generally unsatisfactory to describe most microbial inactivation behavior (Haas and Karra 1984a). The Series-event model describes shoulder behavior when l is greater than unity. Although it is possible to describe tailing behavior using a number of Multiple series-event models, each corresponding to a different clump size L, the complexity in doing so limits the applicability of the Series-event model.

2.3 Inactivation kinetics under disinfectant demand conditions

All of the models discussed thus far from differential rate laws for batch reactors assume that the disinfectant concentration is constant throughout the reactor and equal to the concentration in the reactor effluent. Disinfectant demand-free conditions are unlikely for most oxidants and natural waters (Fair et al. 1948; Hoff 1987). This violates the assumption of a constant disinfectant concentration necessary to derive the Chick-Watson model from which the CT concept originates. To remedy this problem, the SWTR specifies that the characteristic disinfectant concentration be the effluent concentration from the reactor and the characteristic time[2], T_{10}, be found from tracer studies (Lev and Regli 1992a; Teefy and Singer 1990). The use of T_{10} as the characteristic contact time ensures compliance with SWTR required levels of inactivation by indirectly accounting for non-ideal reactor flow conditions (Lev and Regli 1992b; Teefy and Singer 1990).

In specifying that the effluent disinfectant concentration be considered a constant residual over the entire contact time, the SWTR approach may underestimate the level of inactivation. Lawler and Singer (1993) demonstrated for chlorine how this approach can lead to over design of disinfection systems and advocated inclusion of a chlorine decay rate constant into the Chick-Watson model. This remedies the inadequacy of the SWTR approach provided that microbial inactivation behavior adheres to first-order kinetics. For survival curves characterized by shoulder and/or tailing-off effects, a robust kinetic model that incorporates a disinfectant decay rate constant is necessary to ensure that the calculated disinfection design criteria is valid and accurate. This approach is directly

[2] Characteristic time, T_{10}, is the minimum exposure time for at least 90% of the reactor contents.

applicable to reactors with perfect plug flow characteristics in which a disinfectant such as chlorine is added as a stock feed solution near the reactor inlet and decreases in concentration as the reactive segment flows through the reactor (Ley and Regli 1992a).

Decreases in chemical disinfectant residuals in natural waters are attributed to demand caused by particulates, inorganic constituents such as Fe^{2+} and NO_2^-, organic matter, microorganisms, volatilization and reaction of the disinfectant with water, and pH and temperature characteristics (Fair et al. 1948; Hoff 1987; Sobsey 1989). First-order kinetics can generally describe the decomposition of chlorine species (particularly in high demand waters), chlorine dioxide, and ozone in aqueous solution, once the initial demand has been satisfied (Haas and Karra 1984b; Hoigné and Bader 1994; Masschelein 1982; Teefy and Singer 1990). First-order decay is described by:

$$C = C_o \exp(-k't) \qquad (2\text{-}14)$$

where C and C_o are the disinfectant residuals, mg/L, at time t and time zero, respectively, and k' is the first-order decay rate constant, min^{-1}. The rate at which a disinfectant residual decreases is a function of the disinfectant and water characteristics but it is considered to be independent of the microorganisms present (Anmangandla 1993).

2.3.1 Generalized differential rate law

The generalized inactivation rate law, from which the Chick-Watson, Hom, and Rational models were derived for demand-free conditions, can be modified to account for disinfectant demand conditions. Assuming that the chemical disinfectant decay follows first-order kinetics, equation 2-14 can be substituted into equation 2-1 to give the generalized inactivation rate law:

$$\frac{dN}{dt} = -kmN^{x}C_o^{n}e^{-k'tn}t^{m-1} \qquad (2\text{-}15)$$

This differential rate law can be integrated as before to give various kinetic models which account for first-order residual decay and are applicable to batch and perfect plug flow reactors. Disinfection kinetic models derived for demand-conditions are summarized in Table 2-1. Although it may be possible to redefine the Multiple-target and Series-event models, the assumption of simultaneous hits and / lethal reactions being first-order with respect to concentration, respectively (Haas and Karra 1984a; Wei and Chang 1975), make them unsuitable for describing microbial inactivation kinetics (Scheible 1987; Wei and Chang 1975).

2.3.1.1 Chick-Watson model

Assuming x=1 and m=1 as before, the generalized rate law gives a Chick-Watson rate law with disinfectant decay:

$$\frac{dN}{dt} = -kNC_o^{n}e^{-k'tn}$$

This formulation is similar to a differential equation discussed by Hiatt (1964) describing photodynamic inactivation of $T4Br^{+}$ coliphage by proflavine which undergoes irreversible oxidation at a rate independent of the inactivation action. Integration of the modified Chick-Watson rate law gives:

$$\int_{N_o}^{N}\frac{dN}{N} = -kC_o^{n}\int_{0}^{T}e^{-k'tn}dt$$

$$\log\frac{N}{N_o} = -\frac{k}{k'n}(C_o^{n} - C_f^{n}) \qquad (2\text{-}16)$$

2.3.1.2 Hom models

Integration of equation 2-15 assuming x=1 gives the empirical Integral Hom model:

$$\log\frac{N}{N_o} = -kmC_o^n\int_0^T e^{-k'tn}t^{m-1}dt$$

This integral can be numerically approximated with a Riemann sum based on appropriately small Δt partitions (Finch et al. 1993b):

$$\log\frac{N}{N_o} = -kmC_o^n\sum_{\kappa=1}^{z}(e^{-k'tn})_\kappa t_\kappa^{m-1}\Delta t_\kappa \qquad (2\text{-}17)$$

where z is the total number of partitions; κ is the index of summation; and Δt is the time increment partition, the cumulative sum of which equals the total disinfectant contact time. As the number of partitions increases, the accuracy of the numerical approximation improves (Gillett 1984). This method yields parameter estimates of acceptable precision provided that increments of integration, Δt, are sufficiently small. For m less than 0.2, the increment of integration should be less than 10^{-10} min.

A closed-form Hom model which accounts for first-order disinfectant decay can be derived using the Incomplete gamma function (Haas and Joffe 1994):

$$\gamma(\alpha,x) = \int_0^x e^{-z}z^{\alpha-1}dz \qquad \alpha \geq 0,\ x \geq 0 \qquad (2\text{-}18)$$

Figure 2-2 is a contour plot of the Incomplete gamma function generated using Maple V (Waterloo Maple Inc.) and the contouring feature of Transform 3.0 (Fortner Research Inc.). The Incomplete gamma is related to the gamma function as shown in Figure 2.3 in which (Deming 1944):

$$\Gamma(\alpha) = \int_0^\infty e^{-z} z^{\alpha-1} dz \qquad \alpha > 0$$

$$\Gamma(\alpha, x) = \int_x^\infty e^{-z} z^{\alpha-1} dz$$

Tabulated incomplete gamma ratios can be found elsewhere (Harter 1964; Khamis and Rudert 1965). Using the Incomplete gamma function, the Hom rate law (equation 2-15) can be integrated as follows:

$$\frac{dN}{d(nk't)} = \frac{-kmNC_o^n e^{-k'tn} t^{m-1}}{nk'}$$

letting x=z=nk'T

$$\int_{N_o}^{N} \frac{dN}{N} = -kmC_o^n \int_0^x \frac{e^{-z} t^{m-1}}{nk'} dz$$

$$\ln \frac{N}{N_o} = \frac{-kmC_o^n}{nk'} \int_0^x e^{-z} t^{m-1} dz$$

since $T = \frac{z}{nk'}$

$$\ln \frac{N}{N_o} = \frac{-kmC_o^n}{nk'} \cdot \frac{1}{(nk')^{m-1}} \int_0^x e^{-z} z^{m-1} dz$$

$$\ln \frac{N}{N_o} = \frac{-kmC_o^n}{(nk')^m} \int_0^x e^{-z} z^{m-1} dz$$

and substitution gives the Incomplete gamma Hom model:

$$\log \frac{N}{N_o} = \frac{-kmC_o^n}{(nk')^m} \cdot \gamma(m, nk'T) \qquad m > 0,\ nk'T \geq 0 \qquad (2\text{-}19)$$

The Incomplete gamma function is available in the Numerical Recipes Function Pack for Mathcad Plus 6.0 (Mathsoft, Inc.).

Besides the numerical approach there are two analytic approximations of the Hom model. The Efficiency factor Hom model advanced by Haas and Joffe (1994) and the C_{avg} Hom model previously used by Finch et al. (1993a). The Efficiency factor Hom model is stated as (Haas and Joffe 1994):

$$\log\frac{N}{N_o} = -kC_o^n T^m \eta \qquad \text{where} \qquad \eta = \left[\frac{1-\exp\left(-\frac{nk'T}{m}\right)}{\left(\frac{nk'T}{m}\right)}\right]^m \tag{2-20}$$

This approximation is satisfactory provided that m is greater than 0.4. As m approaches zero the efficiency factor is not a valid approximation of the exact solution given by the Incomplete gamma function because it approaches a singularity, tending to infinity. The assumption that model parameter estimates are independent of the water characteristics appears to be valid for resistant organism, such as *Giardia* cysts, based on the reported findings of Haas et al. (1994). *Giardia* cysts suspended in phosphate and phosphate-carbonate buffered laboratory waters were exposed to chemical disinfectants and Efficiency factor Hom parameters estimated for a particular disinfectant. These laboratory water derived models were able to predict levels of *Giardia* cyst inactivation observed in other disinfection trials conducted using natural waters (Haas et al. 1994). For the less resistant organisms *E. coli* (ATCC 13706) and MS2 bacteriophage (ATCC 15597-B1), survival ratios observed in natural waters differed significantly from survival ratios predicted using the Hom-type model with parameter estimates derived from laboratory water disinfection trials (Haas et al. 1994). For resistant organisms, such as encysted

protozoa, model parameter estimates derived from batch disinfection studies conducted using laboratory water may be applicable to natural waters.

The C_{avg} Hom model is based on the concept of a geometric mean residual given by:

$$C_{avg} = \sqrt{C_o \cdot C_f}$$

where C_o and C_f are initial and final disinfectant residuals, respectively. This approximation assumes that disinfectant decomposition follows first-order kinetics and that the disinfectant concentration at the end of the contact time is appreciably greater than zero. The Hom rate law, equation 2-4, can be integrated as before with C^n replaced with C^n_{avg} to give:

$$\log \frac{N}{N_o} = -kC^n_{avg}T^m \qquad (2\text{-}21)$$

2.3.1.3 Rational model

A Rational model which accounts for first-order disinfectant decomposition is given by integrating the generalized rate law, assuming m=1, as follows:

$$\int_{N_o}^{N} \frac{dN}{N^x} = -kC_o^n \int_0^T e^{-k'tn} dt$$

where: $x \neq 1$

$$N^{1-x} - N_o^{1-x} = (x-1) \cdot \frac{k}{k'n}(C_o^n - C_f^n)$$

$$\left(\frac{N}{N_o}\right)^{1-x} = 1 + (x-1) \cdot \frac{k}{k'n}(C_o^n - C_f^n) \cdot N_o^{x-1}$$

$$\log\frac{N}{N_o} = -\frac{\log\left[1+(x-1)\cdot\frac{k}{k'n}(C_o^n - C_f^n)\cdot N_o^{x-1}\right]}{(x-1)} \qquad (2\text{-}22)$$

2.3.1.4 Hom-Power law model

The generalized rate law can be integrated with parameters m and x both present to give a (γ) Hom-Power law model. This model was derived as a hybrid of the Incomplete gamma Hom and Rational model.

$$\int_{N_o}^{N}\frac{dN}{N^x} = -kmC_o^n\int_0^T e^{-k'tn}t^{m-1}dt$$

where: $x \neq 1$

$$N^{1-x} - N_o^{1-x} = (x-1)\cdot\frac{kmC_o^n}{(nk')^m}\cdot\gamma(m,nk'T)$$

$$\left[\frac{N}{N_o}\right]^{1-x} = 1+(x-1)\cdot\frac{kmC_o^n}{(nk')^m}\cdot\gamma(m,nk'T)\cdot N_o^{x-1}$$

$$\log\frac{N}{N_o} = -\frac{\log\left[1+(x-1)\cdot\frac{kmC_o^n}{(nk')^m}\cdot\gamma(m,nk'T)\cdot N_o^{x-1}\right]}{(x-1)} \qquad m>0,\ nk'T\geq 0 \qquad (2\text{-}23)$$

Alternate derivations are possible using the Efficiency factor Hom approximation or by assuming minor disinfectant demand and rearranging the rate law as described elsewhere (Anotai 1996). Note that the number of parameters in the Hom-Power law model makes it prone to overparametrization.

2.3.2 Selleck model

Assuming that the decomposition of a disinfectant is governed by irreversible first-order kinetics, independent of the rate of organism inactivation, the Selleck rate law can be expressed as (Haas 1979):

$$\frac{dS}{dt} = -\frac{nC_o \exp(-k't)}{k} S^{(n+1)/n} \tag{2-24}$$

Integration of this rate law gives a Selleck model which accounts for first-order disinfectant decay:

$$\int_1^S \frac{dS}{S^{(n+1)/n}} = -\frac{nC_o}{k}\int_0^T e^{-k't}dt$$

$$-n(S^{-1/n}+1) = -n\frac{C_o}{kk'}(1-e^{-k'T})$$

$$S = \left[1+\frac{C_o}{kk'}(1-e^{-k'T})\right]^{-n}$$

$$\log\frac{N}{N_o} = -n\log\left[1+\frac{C_o}{kk'}(1-e^{-k'T})\right] \tag{2-25}$$

2.4 Assessment of kinetic models

The adequacy of the various models to describe inactivation kinetics characterized by tailing-off phenomena was evaluated using an unpublished data set for ozone disinfection of heterotrophic plate count (HPC) bacteria. HPC bacteria have been used in several studies on the disinfection efficiency of free chlorine and combined chlorine (Ridgeway and Olson 1982; LeChevallier et al. 1984; Wolfe et al. 1985; Means et al. 1986; LeChevallier et al. 1988, 1992; Pernitsky et al. 1995). A common conclusion from

these studies was that a large proportion of the HPC bacteria population were quickly killed but there is a sub-population of bacteria that are very difficult to inactivate. The kinetic plots are characterized by a "tailing-off" appearance similar to those seen in studies of ozone inactivation of *Giardia* spp. and *C. parvum* (Labatiuk et al. 1992; Finch et al. 1993a, b).

HPC bacteria were maintained in the laboratory according to the methods of Pernitsky et al. (1995). The disinfection protocols of Finch et al. (1993a) were used to expose HPC bacteria suspended in batch reactors to aqueous ozone. Experimental data are summarized in Table 2-2. A maximum likelihood approach was used to estimate parameters for the various kinetic models as described in Chapter 3. The Minerr function of Mathcad Plus 6.0 was used to maximize the logarithm of the likelihood function, lnL. Table 2-3 lists parameter estimates for the models as well as the standard deviation of the errors, $\hat{\sigma}$. For the Rational model and Hom-Power law models, the N_o term was treated as a nuisance parameter and estimated simultaneously with the parameters. A smaller $\hat{\sigma}$ corresponds to a better model fit (Klepikov and Sokolov 1961; Shenton and Bowman 1977). Kinetic models can be ranked in order of decreasing $\hat{\sigma}$ values as follows: Chick-Watson (n=1), Chick-Watson, Rational, Selleck, Hom-Power law, analytic Hom approximations, numerical Hom approximations, and Incomplete gamma Hom (I.g.H.) model. Note that the numerical Hom approximation and I.g.H. model give identical results when a sufficiently small partition size is used in the numerical integration.

Because Chick-Watson kinetics do not adequately describe tailing-off behavior in disinfectant demand-free conditions, model inadequacy under demand-conditions is not

surprising. In comparison, the Selleck model provides a significant improvement to model fit with the same number of parameters. The Rational model further demonstrates that the structure of the inactivation rate law is more critical to model fitting than the number of parameters. With four parameters, including the nuisance parameter N_o, the Rational model is inferior to the two parameter Selleck model. The five parameter Hom-Power law model is an improvement but less robust model than the various three parameter Hom models for describing tailing-off phenomena. The Incomplete gamma Hom model provides an improved fit compared with the analytic approximations. The Incomplete gamma Hom model residuals for the HPC data set were found to be randomly distributed, with approximate zero means, constant variance, and they were consistent with a normal distribution.

The various models were used to calculate survival ratios for HPC bacteria given an initial ozone residual of 0.5 mg/L and a first-order ozone decay rate constant of 0.2 min^{-1}, at 20°C, as illustrated in Figure 2-4. The Chick-Watson model with n fixed at unity, as assumed by the SWTR CT approach, significantly underestimates levels on inactivation throughout the contact time which would lead to overdesign of contactors. The Chick-Watson model with n not fixed to unity introduces the possibility of underdesign because it overestimates levels of inactivation for contact times greater than 15 min. Although the other models are comparable in terms of predicted survival ratios, the Incomplete gamma Hom model is the preferred choice because it does not have the awkward N_o nuisance parameter, like the Rational and Hom-Power law models, and it is applicable to a wider variety of survival curves than the Selleck model.

2.5 Application of kinetic models

The SWTR CT approach may over- or underestimate disinfection requirements because the Chick-Watson model does not adequately describe deviations from first-order kinetics nor does it account for a declining disinfectant residual. Figure 2-5 illustrates the initial ozone residual and contact time required for 99 and 99.9% inactivation of HPC bacteria at 20°C for first-order ozone decay rates ranging from 0.01 to 1.0 min^{-1} based on the Incomplete gamma Hom model. This model offers accurate C_o and T settings whose complex interdependence can be simplified graphically as a process design chart as shown in Figure 2-5 for a given set of conditions such as pH and temperature. Note that it is the unique values of C_o and T that are of interest and not the product of C_o and T. This approach is based on the assumption that the first-order disinfectant decay rate constant is independent of the organism present (Anmangandla 1993) and it is only a function of the water characteristics and disinfectant. Conversely, model parameter estimates are considered independent of the water characteristics and only a function of the disinfectant and organism.

Rather than apply arithmetic safety factors to account for parameter estimate uncertainty, inverse prediction intervals can be calculated based on a given disinfection data set. For a mean expected level of inactivation and first-order decay rate constant, inverse prediction intervals can be calculated for a given level of statistical significance as described elsewhere (Seber and Wild 1989). This alternate approach based on Incomplete gamma Hom calculated C_o and T settings provides a more rational basis for regulatory

design criteria than the current SWTR CT approach for satisfying given levels of pathogen inactivation while minimizing the formation of disinfection by-products.

2.6 Conclusions

Effective control of resistant pathogens in potable water not only requires the use of efficacious disinfectants but also creates the need for optimal design/performance criteria to ensure protection of public health and to minimize risk from exposure to disinfection by-products. The SWTR Guidance Manual (USEPA 1990) provides CT values for several disinfectants which specify the level of inactivation credited based on calculated CT values. In specifying that the effluent disinfectant concentration be considered a constant residual during the total contact time, the SWTR underestimates the Chick-Watson predicted level of inactivation. Lawler and Singer (1993) advocated inclusion of a first-order disinfectant decay rate constant into the Chick-Watson model. Incorporating disinfectant decay into an adequate model confounds the over/under design issue. Hom-type models adequately described the HPC disinfection data which was characterized by "tailing-off" behavior. The Incomplete gamma Hom model is recommended for the design of contactor systems to ensure that treatment requirements are satisfied and that formation of disinfection by-products is minimized.

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Table 2-1. Summary of kinetic inactivation models

Model	Demand-free conditions: $\log\frac{N}{N_o}=$	First-order disinfectant decay: $\log\frac{N}{N_o}=$
Chick-Watson	$-kC^nT$	$-\frac{k}{k'n}(C_o^n-C_f^n)$
(Integral) Hom	$-kC^nT^m$	$-kmC_o^n\sum_{\kappa}^{z}(e^{-k'tn})_\kappa t_\kappa^{m-1}\Delta t_\kappa$
Efficiency factor Hom	Not necessary	$-kC_o^nT^m\eta$
C_{avg} Hom	Not necessary	$-kC_{avg}^nT^m$
Incomplete gamma Hom	Not necessary	$\frac{-kmC_o^n}{(nk')^m}\cdot\gamma(m,nk'T)$
Rational	$-\frac{\log\left[1+N_o^{x-1}(x-1)kC^nT\right]}{(x-1)}$	$-\frac{\log\left[1+(x-1)\cdot\frac{k}{k'n}(C_o^n-C_f^n)\cdot N_o^{x-1}\right]}{(x-1)}$
Hom-Power law	$-\frac{\log\left[1+N_o^{x-1}(x-1)kC^nT^m\right]}{(x-1)}$	$-\frac{\log\left[1+(x-1)\cdot\frac{kmC_o^n}{(nk')^m}\cdot\gamma(m,nk'T)\cdot N_o^{x-1}\right]}{(x-1)}$
Selleck	$-n\log\left[1+\frac{CT}{k}\right]$	$-n\log\left[1+\frac{C_o}{kk'}(1-e^{-k'T})\right]$
Multiple-target	$\log\left[1-(1-e^{-kCT})^{n_c}\right]$	Not derived
Series-event	$\frac{-kCT+\ln\left[\sum_{\kappa=0}^{l-1}\frac{(kCT)^\kappa}{\kappa!}\right]}{\ln(10)}$	Not derived
Multiple series-event	$\log\left[1-\left(1-e^{-kCT}\sum_{\kappa=0}^{l-1}\frac{(kCT)^\kappa}{\kappa!}\right)^L\right]$	Not derived

Source: Chick (1908); Watson (1908); Kimball (1953); Hom (1972); Majumdar et al. (1973); Wei and Chang (1975); Selleck et al. (1978); Haas (1979); Severin et al. (1984); Finch et al. (1993b); Lawler and Singer (1993); Haas et al. (1994); Haas and Joffe (1994); Anotai (1996).

Table 2-2. Data set for ozone inactivation of HPC bacteria in pH 6.9, 0.05 M, phosphate buffer at 20°C

Initial population of HPC bacteria N_0	Applied ozone dose mg/L	Initial ozone residual, C_0, mg/L	Final ozone residual, C_f, mg/L	Applied contact time, T, min	First-order ozone decay rate, k', min^{-1}	Final population of HPC bacteria, N	Observed survival ratio, log-units
3.74×10^6	0.84	0.75	0.19	9.75	0.15	6.36×10^3	-2.8
6.86×10^6	2.05	1.95	1.85	0.58	0.02	3.68×10^4	-2.3
5.28×10^6	1.19	1.12	0.24	14.78	0.12	7.05×10^2	-3.9
5.35×10^6	0.33	0.30	0.08	14.93	0.09	6.13×10^3	-2.9
2.29×10^6	0.31	0.29	0.13	9.87	0.08	6.35×10^3	-2.6
2.55×10^6	0.53	0.53	0.38	4.67	0.08	6.45×10^3	-2.6
4.33×10^6	1.00	1.01	0.62	9.80	0.06	4.06×10^3	-3.0
3.96×10^6	2.13	2.12	1.26	9.53	0.05	$<4.79\times10^2$	<-3.9
5.83×10^4	0.52	0.47	0.45	1.00	0.03	6.38×10^2	-2.0

Notes:

1. The applied ozone dose is the mass of ozone obtained from the stock solution divided by the final volume of the test solution in the reactor.
2. The initial ozone residual is the ozone concentration measured in the reactor following addition of the stock solution.
3. The instantaneous ozone demand is the difference between the calculated applied ozone dose and the measured initial ozone residual.
4. The rate at which aqueous ozone disappears in the reactor solution, based on absorbance measurements at a wavelength of 260 nm, was approximated using a first-order decay expression. The ozone decay rate constant was estimated using the Solver function of Excel 5.0 based on a number of measured ozone residuals in addition to the initial and final measured residual. The absorbance profile for experimental trial listed in the first row of Table 2-2 is provided in Appendix C.
5. Membrane filtration and R2A plate counts were used to enumerate survivors, according to standard method 9215D (Greenberg et al. 1992), following a 7 day incubation at 20°C.

Table 2-3. Summary of parameter estimates for inactivation models describing ozone inactivation of HPC bacteria suspended in pH 6.9, 0.05 M, phosphate buffer at 20°C

Kinetic Inactivation Model	Eq'n	$\hat{k}$	$\hat{m}$	$\hat{n}$	$\hat{x}$	$\hat{N}_o$	$\hat{\sigma}$
Chick-Watson (n=1)	2-16	0.59	-	-	-	-	1.18
Chick-Watson	2-16	0.46	-	0.43	-	-	0.80
Integral Hom	2-17	2.18	0.20	0.22	-	-	0.21
Efficiency factor Hom	2-18	2.19	0.23	0.21	-	-	0.23
C_{avg} Hom	2-19	2.19	0.22	0.20	-	-	0.23
Incomplete gamma Hom (I.g.H.)	2-21	2.22	0.19	0.21	-	-	0.21
Rational	2-22	8.59×10^{-3}	-	0.57	1.51	1.6×10^{6}	0.33
Hom-Power law	2-23	0.34	0.39	0.43	1.24	1.5×10^{6}	0.23
Selleck	2-25	2.70×10^{-2}	-	1.42	-	-	0.25

Note: - denotes not applicable.

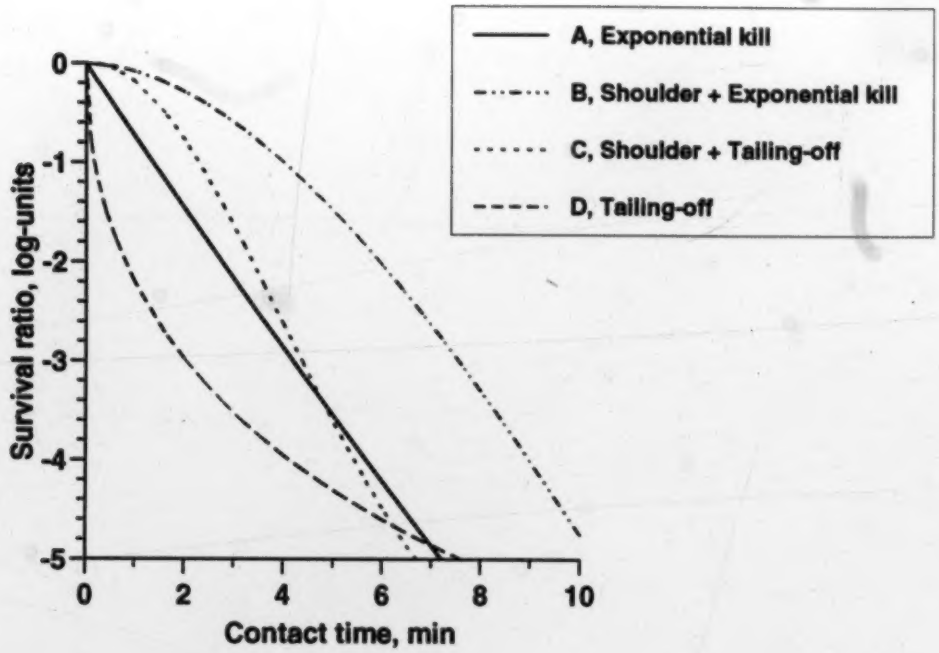

Figure 2-1. Typical microbial survival curves

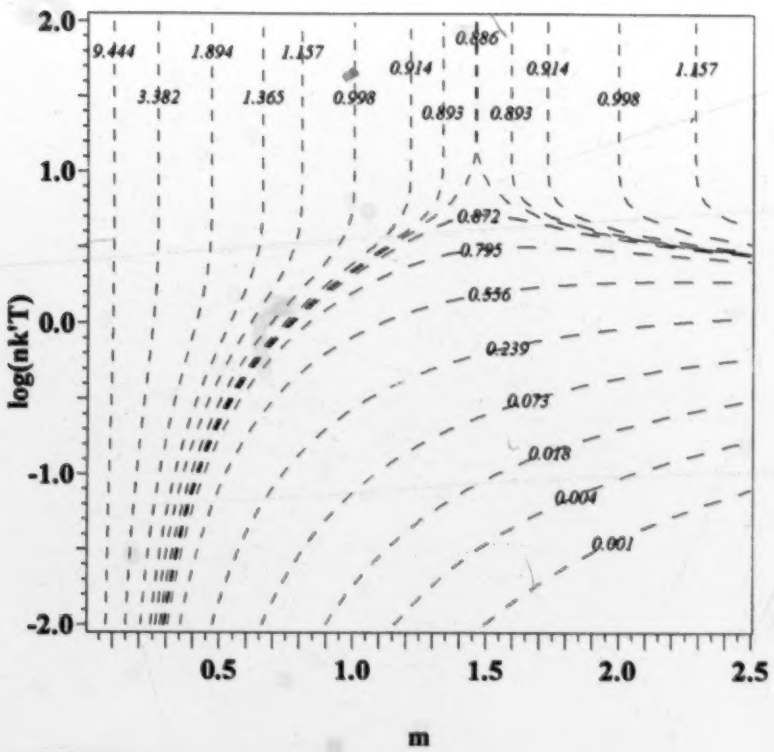

Figure 2-2. Contour plot of the Incomplete gamma function, $\gamma(m,nk'T)$

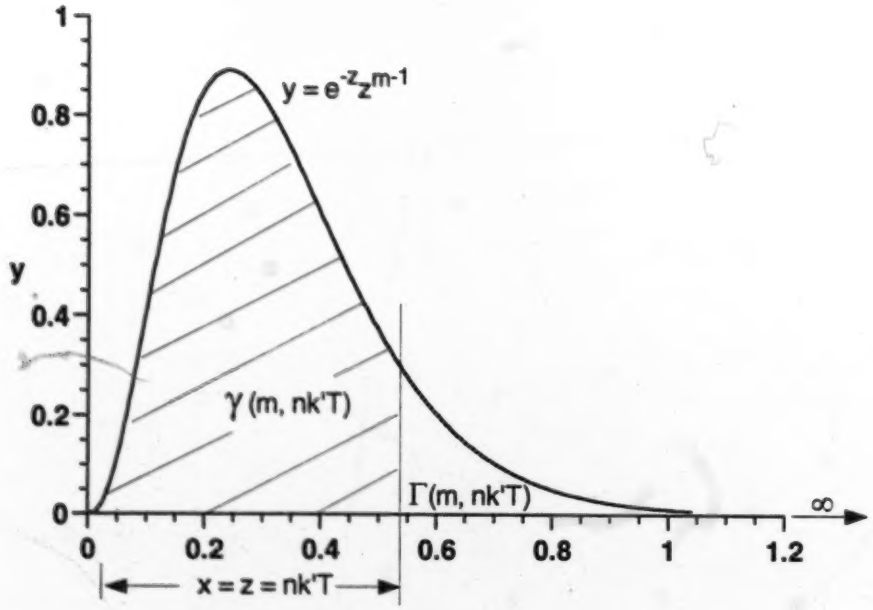

Figure 2-3. The Incomplete gamma function, $\gamma(m,nk'T)$

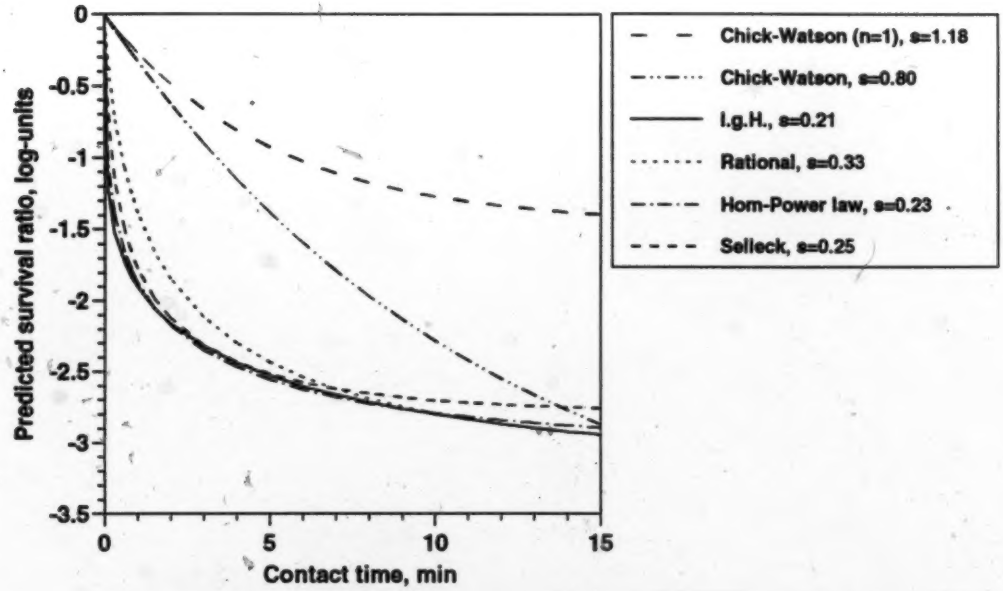

Figure 2-4. Survival ratios of HPC bacteria predicted for an initial ozone residual of 0.5 mg/L and first-order decay rate constant of 0.2 min^{-1} at pH 6.9 and 20°C. Note that $\hat{\sigma}$ is represented by s in the figure legend.

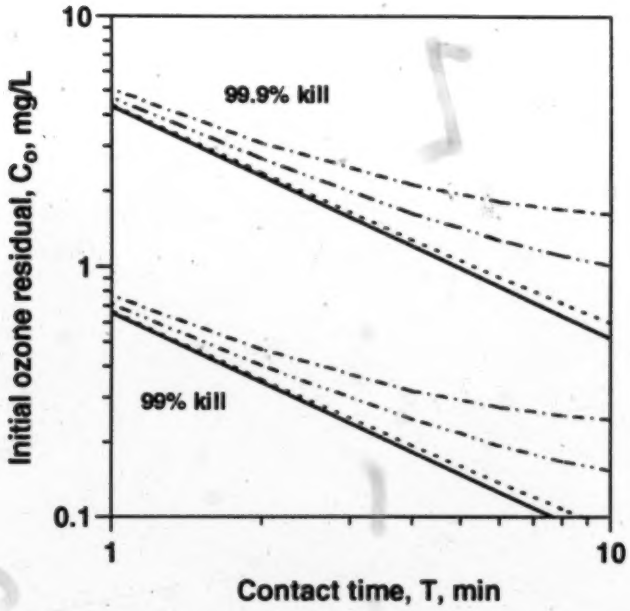

Figure 2-5. Ozone process design chart for 99 and 99.9% inactivation of HPC bacteria at 20°C for first-order ozone decay rates of 0.01, 0.1, 0.5 and 1.0 min^{-1}

Chapter 3

Modeling Chlorine Inactivation Requirements of *Cryptosporidium parvum* Oocysts[1]

3.1 Introduction

Chlorine is the most widely used chemical water disinfectant in the world. Elemental chlorine (Cl_2), hypochlorous acid (HOCl), and hypochlorite ion (OCl^-) are collectively referred to as free (available) chlorine (FAC). Above pH 2, HOCl and OCl^- are the major chlorine species (Snoeyink and Jenkins 1980). It has long been known that HOCl and OCl^- are the active agents in free chlorine, with HOCl being more effective (Fair et al. 1948). Early research on disinfection of *Cryptosporidium* spp. reported that chlorine bleach solutions had little effect on viability of oocysts (Campbell et al. 1982; Ransome et al. 1993; Smith et al. 1988; Sundermann et al. 1987). These studies typically used chlorine bleach concentrations in excess of 1,000 mg/L to affect less than one log-unit inactivation. Another study suggested that neither free chlorine nor monochloramine were practical for a water treatment plant (Korich et al. 1990a).

Laboratory studies have demonstrated that chloramines are less effective than free chlorine for inactivating bacteria, viruses, and protozoa. Some field studies, however, have shown that chloramines are as effective as chlorine in controlling coliform and total bacterial counts when sufficient contact times are provided (Selleck et al. 1978). The biocidal effectiveness of chloramines observed in field studies renewed interest in the use of chloramines as an alternative primary disinfectant (Means et al. 1986). A comparison of these studies suggests that chloramines generated under field conditions, a sequential

[1] Gyürék, L.L., G.R. Finch, and M. Belosevic. (1997). *Journal of Env. Eng., ASCE*, 123(9), 865-875.

chlorine-ammonia process, are somewhat more efficacious than those that are preformed. In both the chlorination of non-nitrified wastewaters and the post-ammoniation of drinking water, there is an initial exposure to free chlorine of short duration followed by a much longer duration of exposure to combined chlorine.

3.2 Materials and Methods

3.2.1 Oxidant Demand-Free Materials

All glassware was initially cleaned using a detergent specifically designed for laboratory glassware and was washed in deionized distilled water with a acetic acid rinse followed by two more high temperature rinses with deionized water (Finch et al. 1987). After the initial cleaning all glassware was rinsed three times with deionized water including one more acetic acid rinse. From then on, all glassware was cleaned using hot deionized water including an acetic acid rinse.

Oxidant demand-free water was prepared by ozonating deionized laboratory water from either a Milli-Q® system (OM-140, Millipore Corp., Bedford, Mass.) or Elga® Ultra-pure system (FisherScientific Limited, Pittsburgh, PA) for at least 30 min, then boiled for at least 10 min. Oxidant demand-free glassware was prepared by exposing acid-washed glassware to laboratory water with an ozone residual of approximately 20 mg/L for at least 3 hours, and storage for at least 2 days at 80°C. Openings in the glassware were covered with aluminum foil to prevent dust from entering the glassware. Ozone gas was generated from extra-dry oxygen using a water-cooled, corona discharge generator (Welsbach T-816, Welsbach Ozone Systems Corporation, Sunnyvale, CA).

Preparation of 0.05 M laboratory buffered water involved dissolution of 0.426 g or 6.818 g of potassium dihydrogen orthoposhate (KH_2PO_4) and 7.098 g or 0.869 g of disodium hydrogen orthophosphate (Na_2HPO_4), AnalaR (BDH Inc.; Toronto, Ontario) reagent grade, into 1 L of laboratory water for pH 8.0 and 6.0, respectively. Buffered laboratory water was similarly made oxidant demand-free using ozone. Oxidant demand-free reactors included 50 mL Nalgene® (Nalge Company, Rochester, NY) polypropylene copolymer centrifugation tubes and 500 and 1,000 mL borosilicate Wheaton Media/Lab bottles. The limited number of oocysts available for experiments throughout the experimental program necessitated the use of the smaller reactors.

It was found that ozone demand-free glassware, lab water, and buffers gave the same result as chlorine demand-free treatment in terms of free chlorine and combined chlorine demand or rate of decomposition. The laboratory and buffered waters were made chlorine demand-free with addition of sodium hypochlorite to provide 5 mg/L as Cl_2, a 2 day contact period under dark conditions, followed by dechlorination using ultraviolet irradiation at 254 nm until chlorine was not detectable based on DPD (N,N-diethylphenylenediamine) colorimetry.

3.2.2 Oxidant Stock Solutions

Chlorine stock solutions were prepared daily as needed using sodium hypochlorite 6% available chlorine solutions (FisherScientific Limited, Pittsburgh, PA or VWR Scientific, West Chester, PA) and oxidant demand-free laboratory water. Monochloramine was formed daily as needed with sodium hypochlorite and ammonium chloride. An ammonium chloride stock was prepared by dissolving 3.819 g, of 105 to 110°C dried ammonium

chloride BDH AnalaR reagent grade, in 1 liter of pH 8 oxidant demand-free 0.05 M phosphate buffer. The sodium hypochlorite and ammonium chloride solutions were diluted using pH 8, 0.05 M, oxidant demand-free phosphate buffer to give 300 mg/L Cl_2 and 100 mg/L NH_4Cl-N. The 300 mg/L chlorine solution was added to an equal volume of the 100 mg/L ammonium chloride solution and mixed for at least 30 min. This produced a monochloramine stock solution with a Cl_2:N weight ratio of 3:1 and an approximate concentration of 150 mg/L as Cl_2.

3.2.3 Residual Oxidant Measurements

The DPD colorimetric procedure was used for assessing chlorine and monochloramine stock solutions (Greenberg et al. 1992). Total and free available chlorine Hach packets were used with oxidant demand-free Fisherbrand® borosilicate glass vials. A standardization curve was prepared daily as needed using potassium permanganate ($KMnO_4$) BDH AnalaR reagent grade and oxidant demand-free glassware. A Pharmacia Biotech Ultrospec 2000 UV/visible and Milton Roy Spectronic 601 were used for spectrophotometry. All standardization curves had coefficients of determination greater than 0.99. Appropriate dilutions were made of high concentration samples to prevent bleaching of the DPD dye. The precision associated with DPD measurements, considered to be <8%, in conjunction with the dilution factor was responsible for some final chlorine residuals found to be apparently greater than the initial residual. This apparent discrepancy in residuals was problematic with the 50 mL Nalgene® reactors because of the small sampling volumes that had to be used for concentration measurements. For trials in which final residuals were apparently greater than initial residuals, it was assumed that the

final concentration was constant over the applied contact time for kinetic modeling purposes.

Forward amperometric titration using a Cl Titrimeter FisherScientific model 397 and phenylarsine oxide was used to measure chlorine species (Greenberg et al. 1992). Chlorine colorimetric DPD measurements were found to be approximately 15% higher relative to amperometry for both free chlorine and monochloramine. This discrepancy in chlorine measurements between DPD and amperometry has been reported elsewhere (Gordon et al. 1992). For preformed monochloramine stock solutions, the fraction of total available chlorine measured as free chlorine, following 30 min of mixing, using the colorimetric DPD and amperometry was 0-3% and 0%, respectively. The rate constant for the kinetic reaction between ammonia and chlorine is 4.2×10^6 $(mol/L)^{-1}s^{-1}$ at 25°C (Morris and Isaac 1983). Based on this value of the rate constant, virtually no free chlorine should coexist with combined chlorine following the first few seconds of mixing in a batch system. For pH 8 oxidant demand-free laboratory water and Cl_2:N weight ratios ≤5:1, monochloramine is the predominant species of inorganic chloramines (combined chlorine) (White 1992). Monochloramine was the only combined species of chlorine detected using colorimetric DPD and amperometry.

3.2.4 Experimental Procedures

For each disinfection trial, an aliquot of the stock oocyst suspension was washed and concentrated before being resuspended in Milli-Q® water. The number of oocysts in the preparation was determined by counting a 1:100 dilution of the working oocyst stock, washed twice with laboratory water, using four complete grids of a hemocytometer.

Oocyst preparations ranging from 1.0×10^6 to 1.5×10^7 were suspended in 25 mL and up to 1,000 mL of oxidant demand-free, 0.05 M, phosphate buffer, at pH 6.0 or 8.0. Oocysts suspended in 50 mL Nalgene® reactors were mixed with a vortex mixer prior to addition of oxidants. Parallel reactors that did not contain oocysts were prepared as necessary for residual oxidant measurements. A Thermix Stirrer was used to mix all reactors except Nalgene® 50 mL reactors which were continuously stirred using a New Brunswick Scientific Benchtop Shaker Model G33 operated at 100 rpm. Experiments were conducted with reactors wrapped in aluminum foil and openings to the reactors covered with aluminum foil to minimize losses of the disinfectant. All experiments were done at 22±1°C. Actual pH values for experimental trials did not deviate by more than ±0.1 pH units.

3.2.4.1 Single Oxidant

Aliquots of stock sodium hypochlorite and preformed monochloramine were applied to reactors using oxidant demand-free tips and Oxford Macro-set and Eppendorf digital pipettes. The colorimetric DPD method was used to measure free chlorine and combined chlorine residuals in experimental and any parallel reactors. Chlorine demand consists of chemical reaction and volatilization demands as well as demand associated with oocysts. For 1,000 mL reactors, chlorine measurements were taken from the reactors containing oocysts and initial chlorine demand was ≤0.1 mg/L. In the absence of oocysts the 50 mL Nalgene® reactors led to relatively higher initial chlorine, chemical reaction, demands. The presence of oocysts in these reactors did not add appreciably to the chlorine demand. The

difference in measured free chlorine residuals in parallel and oocyst containing 50 mL reactors was <0.1 mg/L for both initial and final residual concentrations. Free chlorine and monochloramine decay kinetics were found to be the same for the 50 to 1,000 mL reactors. At the end of the contact time, chlorine species were neutralized using 1M sodium sulfite or sodium thiosulphate BDH AnalaR reagent grade. The weight ratio of Na_2SO_3:Cl_2 was typically in the range of 5:1 to 15:1. This level of excess of reducing agent was demonstrated neither to reduce nor to enhance oocyst infectivity. Another reactor served as a positive control receiving the same dose of reducing agent.

3.2.4.2 Free Chlorine Followed by Monochloramine

Free chlorine conditioning at pH 8 was conducted at applied concentration × time (CT) products ranging from 4 to 11, 20 to 85, and 725 to 2,200 mg·min/L. Applied free chlorine doses for these conditioning bands ranged from 0.2 to 0.6 mg/L, 0.4 to 4.2 mg/L, and 3.5 to 9.2 mg/L, respectively. The concentration of chlorine remaining at the end of the conditioning time was used to calculate the ammonia nitrogen dose necessary for conversion to monochloramine with a 3:1 weight ratio of Cl_2:N. The free chlorine and total chlorine residual were measured 5 to 30 min following addition of ammonium chloride. Following conversion of free chlorine to monochloramine it was necessary to add preformed monochloramine, particularly for those trials involving a chlorine pretreatment level of 4 to 11 mg·min/L, in order to obtain appreciable levels of inactivation. Total and free chlorine residuals were again measured following the addition (if necessary) of any preformed monochloramine stock prior to addition of 1M sodium

sulfite or sodium thiosulphate. Another reactor served as a positive control receiving the same dose of reducing agent. Oocysts exposed to 7.2 mg/L of ammonium chloride for 600 min in independent trials did not reduce nor enhance oocyst infectivity.

Relating observed reductions in viability to monochloramine residuals and contact times vis-à-vis a kinetic model was complicated for those sequential trials involving the addition of preformed monochloramine. The (formed + preformed) monochloramine contact time was the sum of the elapsed time between the addition of stock preformed monochloramine and the reducing agent, and a weighted conversion time term. This term was calculated by dividing the product of the concentration of monochloramine following free chlorine conversion (C_c) × the conversion time (T_c) by a geometric mean residual (C_g), also referred to as C_{avg}, given by:

$$C_g = \sqrt{C_o \cdot C_f} \qquad (3\text{-}1)$$

where C_o is the initial (formed + preformed) monochloramine residual following the addition of stock preformed monochloramine and C_f is the (formed + preformed) monochloramine residual prior to the addition of the reducing agent.

3.2.5 Parasite Methods

Details of the *C. parvum* methods and procedures including production of oocysts, concentration of samples, inoculation into the animal host, and subsequent determination of infection are described in Appendix A. Briefly, oocysts used in this study originate from an isolate obtained by Dr. Harley Moon (National Animal Disease Center). Oocysts were produced in male neonatal Holstein calves (*Bos taurus*). Purified oocysts were stored at 4°C in double deionized water with antibiotics. When required for a dose-

response or disinfection experiment, an aliquot of the stock oocyst suspension was washed twice using Milli-Q® water and concentrated by centrifugation. After disinfection, oocysts suspended in 50 mL Nalgene® reactors were recovered by centrifugation at 27,000 × *g* for 10 min at 4°C. The contents of the 250 and 1,000 mL reactors were transferred to 250 mL Nalgene® polypropylene copolymer centrifugation tubes and recovered by centrifugation at 5,821 × *g* for 20 min at 4°C. Positive control samples concentrated using the two different centrifugation protocols showed no difference in infectivity or *in vitro* excystation. Appropriate dilutions were made and the results of four hemocytometer counts were tested against a Poisson distribution prior to administering a 5 to 10 μL inoculum to four day old CD-1 mice. Two cohorts consisting of a minimum of five CD-1 neonates per cohort were used for each trial. The number of treated oocysts administered per neonate differed by a factor of ten between cohorts.

3.2.6 Interpretation of Infectivity Data

The viability of oocysts following each disinfection trial was analyzed based on a logistic dose-response model (Finch et al. 1993b). The survival ratio (log-units) was estimated from:

$$\log\frac{N}{N_o} = \log\left(\frac{n}{n_o}\right) \tag{3-2}$$

where n is the estimated infectious dose per animal after disinfection and n_0 is the number of oocysts given to each animal. The estimated infectious dose was calculated from a logistic dose-response model developed for each individual batch of oocysts. The logit mean response is given by (Neter et al. 1989):

$$\pi' = \ln \frac{P}{1-P} = \beta_o + \beta_1 X \qquad (3\text{-}3)$$

where P is the proportion of the animal cohort positive for a given inoculum X (log_{10}-units) and β are the logit response model parameters. Model parameters were estimated using the method of maximum likelihood (ML) and Microsoft® Excel® 5.0. ML estimators of β_o and β_1 are those values that maximize the log_e of the likelihood function, L, (Brand et al. 1973; Cox 1970):

$$\ln L = \sum_{i=1}^{a} Y_i(\beta_o + \beta_1 X_i) - \sum_{i=1}^{a} \ln\left[1 + \exp(\beta_o + \beta_1 X_i)\right] \qquad (3\text{-}4)$$

where $Y_i = 0, 1$; and $i = 1, 2, \ldots$, up to a, the number of neonate CD-1 mice used in the dose-response study for a particular batch of oocysts.

This approach gives the reduction of viability of experimental results relative to dose-response trials independent of the viability of the stock oocysts seeded in reactors. Calculation of the estimated infectious dose would be biased if dose-response trials were conducted only at the beginning of the one to three month period of usage and the viability of stock oocysts significantly decreased over that period. To minimize any potential bias caused by decreasing viability of stock oocysts, dose-response trials were spread over the one to three month usage period for each batch of oocysts. The net survival ratio for each experimental trial was calculated by subtracting the survival ratio calculated for the positive control from that of the chemical disinfectant treated group. This accounts for any changes in viability as a result of the oocyst preparation and experimental recovery methodologies.

3.2.7 Kinetic Inactivation Data Analysis

Chemical disinfection results have typically been expressed in terms of a CT product for different levels of inactivation. The CT product can be derived theoretically from the Chick-Watson, pseudo first-order rate law:

$$\frac{dN}{dt} = -kNC^n \tag{3-5}$$

where k is the pseudo-first-order rate constant found experimentally, and n is an empirical constant assumed to be unity. An alternate rate law has been proposed to account for deviations from the Chick-Watson model encountered in practice (Hom 1972):

$$\frac{dN}{dt} = -kmNC^n t^{m-1} \tag{3-6}$$

where m is an empirical constant. Assuming the disinfectant concentration remains constant, integration of equations 3-5 and 3-6 gives the Chick-Watson and Hom models, respectively. However, in many disinfection studies this is an erroneous assumption because the chemical disinfectant may continuously disappear during the contact time.

Although kinetics of disinfectant decomposition in aqueous solution can vary considerably, chlorine and monochloramine decomposition can generally be approximated by first-order kinetics (Qualls and Johnson 1983):

$$C = C_o \exp(-k't) \tag{2-14}$$

where C and C_o are disinfectant residual concentrations at time t and zero, respectively, and k′ is the first-order rate constant, min^{-1}. In this study, the decay of oxidant residuals in 0.05 M phosphate buffer was found to approximate first-order kinetics. Substitution of equation 2-14 into the Hom rate law and integration gives the Integral Hom model:

$$\log\frac{N}{N_o} = -kmC_o^n\int_0^T e^{-k'tn}t^{m-1}dt \qquad (3\text{-}7)$$

Note the only difference between expressing the survival ratio as a base 10 natural logarithm as shown in equation 3-7 and a base e formulation is a factor of ln(10) applied to k parameter. Rather than calculate parameter estimates based on numerical approximations, a closed-form Hom model which accounts for disinfectant decay can be derived using the Incomplete gamma function (Deming 1944):

$$\gamma(\alpha,x) = \int_0^x e^{-z}z^{\alpha-1}dz \qquad \alpha > 0,\ x \geq 0 \qquad (2\text{-}18)$$

to give the Incomplete gamma Hom (I.g.H.) model (Haas and Joffe 1994):

$$\log\frac{N}{N_o} = \frac{-mkC_o^n}{(nk')^m}\cdot\gamma(m,nk't) \qquad m > 0,\ nk't \geq 0 \qquad (2\text{-}19)$$

The Incomplete gamma function is available in various mathematical software packages including Maple V release 3 or Mathcad Plus 6.0 Professional Edition.

This modeling approach is based on a number of assumptions: the rate at which chemical disinfectant residuals disappear in the reactor is not dependent on the presence of microorganisms; the rate of at which the chemical disinfectant disappears is independent of the inactivation reaction; and rate law parameter estimates for an inactivation model are independent of water characteristics.

Kinetic model parameters were estimated using the ML method and the Minerr function of Mathcad Plus 6.0. The $\log_e$ likelihood function is given by (Haas and Jacangelo 1993):

$$\ln L = -v_o \ln\sigma - \frac{1}{2}\sum_{i=1}^{v_o}\left(\frac{y_i - \mu_i}{\sigma}\right)^2 + \sum_{i=v_o+1}^{v} \ln\Phi\left(\frac{y_i^{\leq} - \mu_i}{\sigma}\right) \qquad (3\text{-}8)$$

where v and v_o are the number of trials with inactivation ratios (log-units) greater than infectivity detection limits and non-censored inactivation ratios, respectively; $y_i^{\leq}$ is the infectivity detection limit for trial i; μ_i is the I.g.H. predicted survival ratio for trial i; σ is the standard deviation of the regression errors estimated along with the model parameters, treated as a nuisance parameter, and $\Phi(z)$ is the standard normal distribution given by:

$$\Phi(z) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{z} \exp\left(-\frac{x^2}{2}\right)dx \qquad (3\text{-}9)$$

The Mathcad solution was found to be sensitive to initial parameter estimates. Different sets of initial values were used to validate the global maxima.

Marginal confidence intervals were calculated for model parameter estimates. Upper and lower 95% confidence limits were computed for each of the parameters using the likelihood ratio test (Seber and Wild 1989):

$$\ln L_{1-\alpha}(\beta) \geq \ln L_{max}(\hat{\beta}) - \frac{1}{2}\chi^2_{p,\alpha} \qquad (3\text{-}10)$$

where $\ln L(\beta)$ denotes the ln likelihood and $\ln L_{max}(\hat{\beta})$ its supremum. Mathcad Plus 6.0 was used to compute limits by varying one parameter at a time with the other two parameters fixed to their optimal estimates such that the equality constraint was satisfied.

3.3 Results and Discussion

3.3.1 Model Validity

Table 3-1 provides logit response parameter estimates for different batches of oocysts collected during the course of the experimental program. Marginal confidence limits were constructed for each of the logit parameter estimates using the likelihood ratio test. Viability as measured by infectivity differed significantly among the different batches of oocysts. The infectious dose needed to cause infection in 50% of a cohort, ID_{50}, ranged from 17 to 347 oocysts for the different batches.

Parameter estimates for the various I.g.H. models are provided in Table 3-2 based on the disinfection data summarized in Tables 3-3 to 3-8. Neonate CD-1 mice infectivity data used to calculate observed inactivation ratios are provided in Appendix B. For each model, regression residuals (observed - predicted survival ratios) were plotted against all variables, i.e. predicted and observed survival ratios, contact time, and initial measured oxidant residual. Residual plots indicated that regression errors are randomly distributed, independent of all the variables, with approximate zero means and constant variance. These charts are similar in appearance to Figure 3-1 which is a plot of residuals with respect to predicted survival ratios for the monochloramine model for a chlorine pretreatment of 20 to 85 mg·min/L. A Kolmogorov-Smirnov goodness of fit test was used to examine the normality of the residuals (Haas and Jacangelo 1993). Maximum absolute differences (D) between the calculated Kaplan-Meir product-limit cumulative distribution function and standard normal distribution were calculated and are listed in Table 3-2. These values were compared to critical D values tabulated elsewhere

(Lilliefors 1967). The calculated D value for the monochloramine model with chlorine pretreatment of 725 to 2,200 mg·min/L was significant for an α of 0.15. For all other I.g.H. models, calculated D values were significant for α greater than 0.20. A lack of fit test was also used to detect possible I.g.H. model inadequacy for the monochloramine data set corresponding to a chlorine pretreatment level of 20-85 mg·min/L. Lack of fit and pure experimental error mean squares were calculated to be 0.21 (7 d.f.) and 0.17 (4 d.f.), respectively. A probability value of 0.45 for the ratio of mean squares suggests no lack of fit (Neter et al. 1989). Regression errors for all models are consistent with a normal distribution, maximum likelihood assumptions are satisfied, and hence parameter estimates are valid.

3.3.2 Free Chlorine

Parameter estimates and experimental data for free chlorine at pH 6 are summarized in Tables 3-2 and 3-3, respectively. The I.g.H. model is based on 8 trials with applied chlorine doses ranging from 1.7 to 17 mg/L and contact times from 60 to 1,030 min. Trials 177, 179, 269, 323, and 351 were not used for parameter estimation. Regression residual plots indicated that trial 177 was an outlier. Observed infectivity reductions in trials 269 and 323 were left-censored and could not be used for model fitting. Trial 351 exposed oocysts to 80 mg/L of free chlorine for 120 min resulting in >3 log-units inactivation. The model predicted >10 log-units inactivation. This trial was not included in the parameter estimation and suggests that a modeling discontinuity may exist between 17 and 80 mg/L, below or above which different rate laws apply. Figures 3-2 and 3-3 illustrate the free chlorine kinetic model developed in the present study. The initial

disinfectant residual and contact time required for 0.5 and 1.0 log-unit of *C. parvum* inactivation at pH 6 and 22°C are provided by first-order disinfectant decay rate constants of 0 and 1×10^{-3} min^{-1}.

Experimental data for free chlorine at pH 8 is summarized in Table 3-4. Virtually no inactivation was observed when oocysts were exposed to free chlorine concentrations ranging from 4.9 to 80 mg/L at 22°C, and contact times of 48 to 245 min. A free chlorine dose of 45 mg/L applied for 1,045 min was required to affect >1.9 log-units inactivation. Hom rate law parameters were therefore not calculated for chlorine at pH 8. An OCl^- ion to HOCl relative efficacy ratio was not calculated for *C. parvum*. The I.g.H. model developed for pH 6 was used to calculate infectivity reductions for pH 8 trials based on the fraction of free chlorine as hypochlorous acid, i.e. $C_0 \times 0.28$. For a dissociation constant of 2.6×10^{-8} moles/L at 20°C, hypochlorous acid constitutes 97.5, 79.3, and 27.7% of the free chlorine at pH 6, 7, and 8, respectively (White 1992). A comparison of the observed and predicted values in Table 3-4 indicates that inactivation at pH 8 can be attributed to the equilibrium concentration of hypochlorous acid.

Interestingly for trials 152 and 353 in which oocysts were exposed to 14 and 20 mg/L free chlorine for 15 min at pH 8, infectivity was enhanced relative to controls. Oocysts exposed to between 10 and 15 mg/L of monochloramine for contact times less than 50 min also resulted in enhanced infectivity as shown in Table 3-5. Enhanced excystation following chlorine treatment has been reported elsewhere and has been attributed to thinning, perforation, or complete removal of the outer layer of the oocyst wall (Reduker and Speer 1985). The inner zone of the inner oocyst wall was reportedly unaffected by

sodium hypochlorite treatment. Other studies have shown that hypochlorite treatment sensitizes *Clostridium botulinum* spores to germination by lysozyme. Scanning electron microscopy and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that spore coat proteins may be removed by hypochlorite treatment, increasing permeability to the cortex (Foegeding and Busta 1983).

There were few data found in the literature that investigated the effect of chlorine on the infectivity of *C. parvum* (Fayer 1995; Korich et al. 1990b). Korich et al. (1990b) collected data from bench-scale experiments in which *C. parvum* oocysts were suspended in pH 7 oxidant demand-free, 0.01 M, phosphate buffer at 25°C. The oocysts were exposed to 80 mg/L of chlorine for 30, 60, 90, and 120 min. Neonatal BALB/c mice infectivity was used to estimate inactivations of 0 to 1, <2, 3 to 4, and >4 log-units, respectively. These findings agree with the pH 6 data of the present study in which exposure of oocysts to 80 mg/L of free chlorine for 120 min resulted in >3 log-units inactivation. Fayer (1995) suspended *C. parvum* oocysts in 1.31, 2.63, and 5.25% aqueous sodium hypochlorite (Clorox laundry bleach) with contact times ranging from 10 to 120 min at 21°C. Histology scores of neonatal BALB/c mice showed no reduction in infectivity for any of the experimental conditions. Commercial bleach solutions are typically in excess of pH 12. These findings suggest that hypochlorous acid is a significantly better chlorine species for inactivation of *C. parvum* than the hypochlorite ion. The hypochlorite ion is a relatively poor disinfectant, presumably because of it's negative electrical charge which inhibits it's diffusion across biological membranes.

Most of the literature dealing with the effects of chlorine species on protozoan cysts involves free chlorine and its effect on *Giardia* cysts. Using *in vitro* excystation, Jarroll et al. (1981) showed that inactivation of *G. lamblia* cysts exposed to free chlorine decreased as the pH rose from 6 to 8 for the same chlorine concentration and temperature. At 5°C, a chlorine concentration of 8 mg/L inactivated >2.7 log-units of the cysts at pH 6 and 7, but required 30 min to achieve the same result at pH 8 (Jarroll et al. 1981). In a similar study, *G. lamblia* cysts exposed to 2.5 mg/L of chlorine at 5°C provided >2 log-units inactivation for a 30 minute contact time at pH 6, whereas a 60 minute contact time was required at pH 7 and 8 (Rice et al. 1982). Free chlorine was shown to be more effective cysticide for *E. histolytica* at lower pH where hypochlorous acid predominates (Chang and Fair 1941; Stringer and Krusé 1971). The phenomenon of increasing effectiveness of free chlorine with increasing pH has been reported for *Giardia* cysts (Leahy et al. 1987) and viruses (Engelbrecht et al. 1980). Alterations to the surface of cyst walls at alkaline pH was considered a possible explanation to this anomaly (Rubin et al. 1989). This is supported by the enhanced efficacy of chlorine dioxide to inactivate *Naegleria gruberi* at pH 9 compared with pH 5 and 7, at 25°C, because chlorine dioxide does not dissociate nor disproportionate into different chemical species within this pH range (Sproul et al. 1983). However, for free chlorine this anomalous disinfection behavior at pH >9 is attributed to chemical ion-pair interactions (Haas 1981). Hypochlorite ion-pair formation can be neglected in the present study because a significant fraction of hypochlorous acid exists at pH 8. For *C. parvum* oocysts, the data from this study suggest that cyst wall permeability is the same from pH 6 to 8.

A free chlorine CT product of 4,800 mg·min/L, which incorporates a safety factor of 2, has been proposed for 1 log-unit inactivation at pH 7, 25°C (Korich et al. 1990b). Extrapolation of this CT product to low dose conditions typical of water treatment is inappropriate for two reasons: the rate law on which the CT product is based, equation 3-5, is generally inadequate; and a modeling discontinuity appears to exist between chlorine concentrations of 20 and 80 mg/L. For comparative purposes, CT values ranging from 1,700 to 4,000 mg·min/L (without a safety factor) can be calculated from Figure 3-3.

The I.g.H. model illustrated in Figures 3-2 and 3-3 can assist in the design and/or evaluation of disinfection systems provided that the disinfectant decay rate constant, k', is known. For example, the pH 6 kinetic model can be used to evaluate the effectiveness of the superchlor-dechlor process. Chlorination followed by dechlorination has been used in Great Britain as an interim solution for *Giardia lamblia* destruction to compensate for inadequate contact times. Given a 100 minute contact time, the free chlorine concentration necessary to provide 0.5 log-unit inactivation can be calculated to be 7 mg/L at 22°C.

3.3.3 Preformed Monochloramine

Parameter estimates and experimental data describing preformed monochloramine inactivation of *C. parvum* oocysts at pH 8 are summarized in Tables 3-2 and 3-5, respectively. The model is based on 9 trials with applied doses ranging from 2.5 to 15.0 mg/L and contact times from 60 to 1,000 min. Trials 113, 283, 285, 339, 341, 343, and 521 were not used for parameter estimation. Observed reduction in infectivity for trial 521 was left-censored. Trial 111 was used for parameter estimation by assuming that the

observed reduction in infectivity was 0. Predicted levels of inactivation for trials 285 and 343 in which oocysts were exposed to 80 mg/L (as Cl_2) of monochloramine were significantly higher than those observed and these trials were therefore not used for model fitting. Trials 283 and 341 found that oocyst infectivity was enhanced following exposure to between 10 and 15 mg/L monochloramine for contact times less than 50 min. This parallels a similar finding for sodium hypochlorite treatment of oocysts. Figures 3-2 and 3-3 illustrate the preformed monochloramine kinetic model.

Earlier work used (preformed) monochloramine at 110 mg/L (as Cl_2) in pH 9 to 10 oxidant demand-free, 0.01 M, phosphate buffer at 25°C (Korich et al. 1990b). The Cl_2:N weight ratio was not provided. Using BALB/c mice, reductions of infectivity of approximately 1 and <1.3 log-units were reported for 30 and 90 minute contact times, respectively. These data are consistent with trial 285 in which 0.6 log-units inactivation of oocysts was produced by exposure to 80 mg/L for 50 min. This agreement in results between studies indicates that *C. parvum* oocyst wall permeability remains relatively constant between pH 8 and 10.

Korich et al. (1990b) proposed a CT product of 7,200 mg·min/L for 1 log-unit inactivation at pH 9-10, 25°C. As discussed above for chlorine at pH 6, extrapolation of Korich's CT product to low chlorine dose conditions may be inappropriate. For comparative purposes, CT values ranging from 3,300 to 7,000 mg·min/L (no safety factor) can be calculated from Figure 3-3.

3.3.4 Free Chlorine versus Monochloramine

Figures 3-2 and 3-3 can be used to compare the relative disinfection efficiency of free chlorine at pH 6 to (preformed) monochloramine at pH 8. For low levels of inactivation, i.e., ≤0.5 log-units, the model suggests that free chlorine is much more efficacious than monochloramine. Considering 1.0 log-unit inactivation, free chlorine disinfection requirements approach those of preformed monochloramine. Conversely, a relatively small increment in the monochloramine dose is necessary to increase levels of *C. parvum* oocyst inactivation from 0.5 to 1.0 log-units. This suggests that a threshold concentration exists for monochloramine. The influence of the decay rate constant, k', to significantly increase monochloramine disinfection requirements, as shown in Figures 3-2 and 3-3, tends to support this threshold hypothesis.

Inactivation studies of biofilm bacteria have shown that monochloramine is as effective as free chlorine (LeChevallier et al. 1988). A recently espoused explanation for the poor efficacy of free chlorine against biofilm microorganisms is that the reaction rate of chlorine with cellular biomass is fast enough that diffusion of this disinfectant into the biofilm readily becomes rate limiting (Chen and Stewart 1996). It is interesting that the kinetic inactivation rate constant for free chlorine is approximately 10^4 times larger compared with preformed monochloramine.

Most laboratory disinfection studies have found that preformed chloramine compounds, especially in waters above pH 7, are less biocidal than free chlorine or formed monochloramine (Ward et al. 1984). Meyer et al. (1989) used *in vitro* excystation to compare the efficacy of pre-ammoniation, using ammonium sulfate followed by sodium

hypochlorite (Cl_2:N weight ratio of 7:1), to that of preformed monochloramine on *Giardia muris* cysts suspended in buffered water at 3 and 18°C. Lower levels of inactivation reported for the preformed monochloramine were attributed to the brief exposure of cysts to free chlorine while chloramines were forming (Meyer et al. 1989). Ward et al. (1984) reported that rates of *Escherichia coli* inactivation using pre-ammoniation at pH 6, 7, and 7.5 were comparable with those of free chlorine. At pH 8, rates of inactivation using pre-ammoniation were similar to those for preformed chloramines. Increased levels of inactivation at pH <7.5 were attributed to the presence of free chlorine for several min, prior to complete chloramine formation (Ward et al. 1984). Berman et al. (1992) similarly reported that the rate of inactivation of *E. coli* and *Klebsiella pneumoniae* was faster for formed monochloramine, generated by simultaneous addition of chlorine and ammonium sulfate to pH 7 buffer at 5°C (Cl_2:N weight ratio of 5:1), compared with preformed monochloramine. At pH 7 and 5°C, 5 min is required for 90% conversion of free chlorine to monochloramine (White 1992).

3.3.5 Free Chlorine Followed by Monochloramine

Sequential trials were grouped according to applied free chlorine CT levels of 4 to 11, 20 to 85, and 725 to 2,200 mg·min/L. Data grouped according to these pretreatment levels provided significantly lower σ values compared with pretreatment levels of 4 to 85 and 20 to 2,200 mg·min/L. A smaller σ value is an indication of model predicted values agreeing more closely with observed survival ratios. Experimental data are summarized in Tables 3-6 to 3-8. Trial 56 was not used for parameter estimation because of excessive chlorine demand and trials 636 and 638 were considered outliers. Trials 132, 155, 275,

276, 509, and 511 were not used for parameter estimation because observed infectivity reductions were left-censored. A cross-validation comparison of observed and predicted values for these trials supports the I.g.H. monochloramine model for a free chlorine pretreatment level of 20 to 85 mg·min/L. Note this data set contains a number of serially correlated experimental trials. Serially correlated observations were obtained from the same reactor at applied contact times of 100, 200, 400 and 1,200 min. With such widely separated sampling times, serial correlation among regression errors is less important (Seber and Wild 1989). Figures 3-4 and 3-5 summarize the initial monochloramine residual and contact times for 0.5 and 1.0 log-unit inactivation of *C. parvum* oocysts at 22°C following pretreatment with free chlorine at pH 8.

Inactivation modeling suggests that the efficacy of monochloramine formed in the post-ammoniation process is greater than that of preformed monochloramine as well as free chlorine at pH 6. Figures 3-3 and 3-5 illustrate that approximately 3.9 mg/L of free chlorine at pH 6, and 2.5 mg/L of (formed) monochloramine at pH 8, corresponding to the 20-85 mg·min/L pretreatment level, are required to provide 1.0 log-unit *C. parvum* inactivation for a contact time of 1,000 min. These findings are not in agreement with several previous laboratory studies, excluding biofilm findings, which reported that free chlorine is more efficacious than chloramines. The greater efficacy of formed relative to preformed monochloramine has commonly been attributed to low levels of free chlorine prior to complete chloramine formation. However, if this were true, levels of inactivation would be expected to decrease with the implementation of post-ammoniation because presumably more efficacious free chlorine would be converted to combined chlorine. The

experimental results of this study suggest that post-ammoniation at pH 8 is more effective in controlling *C. parvum* oocysts than free chlorine alone at pH 6.

Other studies have shown that chloramines generated using either pre- or post-ammoniation are adequate and, in some cases, superior to free chlorine in terms of indicator organism reductions (Wolfe et al. 1985). One field study for example found that chloramines generated using post-ammoniation and a Cl_2:N weight ratio of 4:1 were more effective in reducing total coliforms in a distribution system compared with the use of free chlorine alone at pH 8.5 to 9 (Norman et al. 1980). The conversion of free chlorine to chloramines occurs in hundredths of a second near the optimum pH of 8.3 at temperatures of 20 to 30°C. The pilot-scale chloramination study of Selleck et al. (1978) compared free chlorine with the pre-ammoniation process. To account for the greater bactericidal efficacy observed with pre-ammoniation, it was postulated that highly reactive, short-lived free radicals were produced during the oxidation of ammonia nitrogen (Selleck et al. 1978). To date no data have been found to support the existence of the proposed free radical.

3.3.6 Synergism

Sequential treatment by chemical compounds which provides more inactivation than expected from the additive (logarithmic) effects of single compounds demonstrates synergy. Free chlorine pretreatment levels used in post-ammoniation trials of the present study were shown to not provide any discernible reduction in animal infectivity. If chloramine inactivation were an additive phenomenon, the initial concentration and contact time of monochloramine required for a given level of inactivation would be the

same regardless of the level of free chlorine pretreatment. Figures 3-4 and 3-5 illustrate that increasing levels of free chlorine pretreatment reduce subsequent monochloramine concentrations and contact time necessary for a given level of inactivation.

There have been reports in the literature of synergism between disinfectant species leading to more effective treatment. MacKenzie (reported in Houghton 1950) first observed the greater efficacy of the chloramination process compared with preformed chloramine in 1936, and suggested that initial transient free chlorine, if not itself promoting rapid kill, may act to "devitalize" microorganisms so that they are more readily killed by chloramine. Sequential application of free chlorine followed by monochloramine was found to be an effective viral-inactivating agent (Berman et al. 1992). Ammonium sulfate was added to a 0.05 M, KH_2PO_4, pH 7 buffered water at 5°C containing 2 mg/L free chlorine to form monochloramine (Cl_2:N weight ratio of 5:1). For free chlorine pretreatment involving contact times ≤0.5 min, the level of MS2 coliphage inactivation was greater than that attributable to the effect of free chlorine pretreatment alone.

A study investigating *E. coli* inactivation hypothesized that there is a potential mechanism of synergism consisting of sub-lethal injury caused by free chlorine resulting in enhanced sensitivity to monochloramine (Kouame and Haas 1991). To explain the synergistic nature of the data collected in the present study, it is postulated that free chlorine pretreatment affects the oocyst wall sufficient to alter the permeability but not to provide a measurable reduction in infectivity. Subsequent treatment with monochloramine permits the disinfectant to diffuse more readily through the oocyst wall and damage

sporozoites. It is further postulated that oocyst wall permeability with respect to monochloramine increases proportionally with the level of free chlorine pretreatment.

3.4 Conclusions

Hypochlorous acid is a significantly better chlorine species for inactivation of *C. parvum* compared with hypochlorite ion. *C. parvum* oocyst wall permeability is the same from pH 6 and 10. Extrapolation of Korich's CT value to low chlorine doses typical of water treatment is inappropriate. The findings of this study support the use of chloramines as an alternative primary disinfectant provided appropriate disinfectant concentrations and contact times are used. The use of monochloramine alone is not practical for most utilities because of an apparent threshold concentration. Post-ammoniation at pH 8 is not only more effective than preformed monochloramine but it can also be more efficacious than free chlorine at pH 6. Increasing levels of free chlorine pretreatment proportionally reduce the subsequent monochloramine concentration and contact time necessary for a given level of inactivation. The greater efficacy of formed compared with preformed monochloramine observed in the present study is explained by synergism. Oocyst wall permeability is likely to increase proportionally with respect to the level of free chlorine pretreatment. Additional work is needed to validate these models for water quality conditions found in practice.

3.5 References

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Table 3-1. Logit response modeling of dose-response data per oocyst batch

Trial No.	$\hat{\beta}_o$	$\hat{\beta}_1$	+/- 90% $\hat{\beta}_o$ Limits	+/- 90% $\hat{\beta}_1$ Limits	ID_{50}	No. of CD-1 mice used
1-49	-3.87	3.15	-3.5, -4.2	3.4, 2.9	17	257
56-100	-4.42	2.44	-4.0, -4.8	2.7, 2.2	65	148
101-218	-3.52	1.88	-3.2, -3.9	2.1, 1.7	74	177
219-356	-5.55	2.59	-5.1, -6.0	2.8, 2.4	139	133
357-460	-6.02	2.37	-5.7, -6.4	2.5, 2.2	347	226
473-526	-6.50	3.53	-5.9, -7.1	3.8, 3.2	69	84
527-641	-5.33	3.35	-4.8, -5.9	3.7, 3.1	39	88

Table 3-2. Incomplete gamma Hom model parameter estimates and confidence limits for chlorine compounds

Disinfectant	No. of trials	pH	$\hat{k}$	+/- 95% $\hat{k}$ Limits	$\hat{m}$	+/- 95% $\hat{m}$ Limits	$\hat{n}$	+/- 95% $\hat{n}$ Limits	$\hat{\sigma}$	Model Constraints: C = Conc. (mg/L) T = Time (min)	D
SINGLE:											
Free Available Chlorine	8	6.0	1.1×10^{-2}	1.5×10^{-2}	0.50	0.56	0.80	0.93	0.30	$1.7 \le C \le 17$	0.16
				6.9×10^{-3}		0.42		0.62		$60 \le T \le 1030$	
Preformed Monochloramine	7	8.0	1.1×10^{-6}	1.4×10^{-6}	1.28	1.32	2.53	2.63	0.33	$2 \le C \le 15$	0.21
				7.6×10^{-7}		1.22		2.40		$60 \le T \le 1,000$	
MONOCHLORAMINE (for a given free chlorine pretreatment level):											
4-11 mg·min/L Cl_2	8	8.0	7.1×10^{-5}	8.3×10^{-5}	0.95	0.98	1.63	1.69	0.15	$1 \le C \le 15$	0.19
				5.9×10^{-5}		0.92		1.56		$2 \le T \le 500$	
20-85 mg·min/L Cl_2	14	8.0	1.7×10^{-2}	2.4×10^{-2}	0.51	0.56	0.59	0.81	0.44	$0.8 \le C \le 8$	0.15
				1.1×10^{-2}		0.44		0.24		$10 \le T \le 1,200$	
725-2200 mg·min/L Cl_2	7	8.0	5.3×10^{-2}	6.9×10^{-2}	0.22	0.27	1.31	1.47	0.30	$2.2 \le C \le 6.4$	0.26
				3.9×10^{-2}		0.17		1.15		$10 \le T \le 450$	

Table 3-3. Free chlorine inactivation data of *C. parvum* at pH 6.0 and 22°C

Trial No.	Reactor vol., mL	No. of oocysts in reactor, $\times 10^6$	Applied dose, mg/L	Initial residual, mg/L	Final residual, mg/L	Contact time, min	First-order decay rate constant, k', min^{-1}	Net infectivity reduction, log units	I.g.H. predicted reduction, log units
2	50	1.0	14.7	14.0	13.6	240	1.1×10^{-4}	> 1.8	1.2
12	50	1.1	14.7	†11.6	13.7	60	0	0.6	0.7
14	50	1.1	4.9	†3.5	3.8	240	0	0.6	0.9
120	25	5.0	5.4	3.9	3.8	245	9.5×10^{-5}	0.7	0.5
176	25	5.0	11.2	11.0	11.0	240	0	0.6	1.0
177	25	5.0	16.9	†16.9	17.3	160	0	0	1.2
179	25	5.0	16.9	†16.3	17.5	60	0	-0.6	0.8
268	25	5.0	15.0	14.2	14.1	160	1.3×10^{-5}	1.0	1.0
269	25	5.0	15.0	†13.9	14.1	60	0	< 0.1	0.7
321	25	5.0	15.5	†16.0	16.6	60	0	> 0	0.8
323	25	5.0	10.5	10.9	10.5	240	1.8×10^{-4}	< 0.9	1.1
351	25	5.0	79.8	†73.3	78.2	120	0	> 3.0	NA
519	475	7.5	1.7	1.73	1.6	1032	5.2×10^{-5}	0.5	0.5

Note: NA denotes not applicable.

†Final residuals were considered to apply over the entire contact time for parameter estimation.

Table 3-4. Free chlorine inactivation data of *C. parvum* at pH 8.0 and 22°C

Trial No.	Reactor vol., mL	No. of oocysts in reactor, $\times 10^6$	Applied dose, mg/L	Initial residual, mg/L	Final residual, mg/L	Contact time, min	First-order decay rate constant, k', min^{-1}	Net infectivity reduction, log units	I.g.H. predicted reduction, log units
1	50	1.0	14.7	13.9	13.5	60	5.6×10^{-4}	> 0.4	0.2
3	50	1.0	4.9	3.0	2.7	240	4.0×10^{-4}	> 0.1	0.1
11	50	1.1	4.9	2.2	†3.8	60	0	0.2	0.1
13	50	1.1	14.7	13.0	†13.6	240	0	0.1	0.5
57	25	5.0	6.9	6.2	5.0	240	8.8×10^{-4}	0.3	0.3
121	25	5.0	15.9	15.3	15.2	245	3.5×10^{-5}	0	0.5
152	25	5.0	20.3	20.1	†21.0	15	0	< -1.0	NA
235	25	5.0	80.0	76.2	75.7	48	1.5×10^{-4}	< 0	NA
353	25	5.0	15.0	13.8	†14.2	15	0	-0.3	NA
517	475	7.5	45.0	42.4	40.7	1045	3.9×10^{-5}	> 1.9	2.5

Note: NA denotes not applicable.

Table 3-5. Preformed monochloramine inactivation data of *C. parvum* at pH 8.0 and 22°C

Trial No.	Reactor vol., mL	No. of oocysts in reactor, $\times 10^6$	Applied dose, mg/L	Initial residual, mg/L, Cl_2/ NH_2Cl	Final residual, mg/L, Cl_2/ NH_2Cl	Contact time, min	First-order decay rate constant, k', min^{-1}	Net reduction in infectivity, log-units	I.g.H. predicted reduction, log units
32	50	1.0	10.7	0.2/9.7	0.2/9.1	480	1.2×10^{-4}	1.3	0.8
77	25	5.0	4.8	0/4.4	0/4.4	480	0	0	0.1
79	25	5.0	9.4	0/9.7	0/9.2	480	9.5×10^{-5}	0.2	0.8
111	25	5.0	6.0	0/6.1	0/5.1	60	3.1×10^{-3}	< 0	0
113	25	5.0	15.0	0/15.2	0/15.2	13	0	1.2	NA
114	25	5.0	15.0	0/15.2	0.4/14.2	480	1.4×10^{-4}	2.4	2.5
133	25	5.0	15.0	0.4/14.8	0.4/13.8	480	1.4×10^{-4}	> 2.0	2.3
283	25	5.0	10.5	0.3/10.2	0.2/10.0	46	3.9×10^{-4}	-0.5	NA
285	25	5.0	80.0	2.7/72.7	2.1/75.9	50	0	0.6	NA
339	25	5.0	15.0	0/13.7	0/13.9	50	0	< 0	NA
341	25	5.0	15.0	0.7/13.3	0.7/12.9	5	7.2×10^{-3}	-0.7	NA
343	25	5.0	80.0	1.9/77.0	0.9/68.5	500	2.3×10^{-4}	3.3	NA
521	475	7.5	2.5	0/2.4	02.2	1002	1.0×10^{-4}	< 0.2	0.1

Note: NA denotes not applicable.

Table 3-6. Monochloramine inactivation data for a free chlorine pretreatment level of 4 to 11 mg·min/L at pH 8.0 and 22°C

Trial No.	Reactor vol., mL	No. of oocysts in reactor, $\times 10^6$	Applied Cl_2 dose, mg/L	Final residual, mg/L	Free Cl_2 contact time, min	Contact time prior to adding preformed NH_2Cl, min	Applied dose of preformed NH_2Cl, mg/L	NH_2Cl residual, mg/L, Cl_2/NH_2Cl	Final NH_2Cl residual, mg/L, Cl_2/NH_2Cl	Total contact time of NH_2Cl, min	NH_2Cl first order decay rate constant, k', min^{-1}	Net reduction in infectivity, log units	I.g.H. predicted reduction, log units
184	25	5.0	0.5	0.2	20	30	1.4	0/1.3	0/1.2	520	2.2×10^{-4}	0.2	0
185	25	5.0	0.5	0.2	20	30	15.0	0.4/15.3	0.4/14.7	514	8.1×10^{-5}	2.2	2.2
190	25	5.0	0.5	0.3	8	0	15.0	0.5/15.4	0.3/†15.5	2	0	0.1	0
193	25	5.0	0.5	0.3	7	0	10.0	0.4/10.6	0.2/10.4	240	8.7×10^{-5}	0.5	0.6
274	25	5.0	0.2	0.1	48	0	0.8	0/0.9	0/0.8	60	3.0×10^{-4}	< 0.1	0
276	25	5.0	0.6	0.3	7	3	10.0	0.3/10.1	0.6/9.5	483	1.3×10^{-4}	< 1.6	1.1
365	25	5.0	0.5	0.5	8.5	10	9.6	0/10.0	ND	492	0	1.3	1.1
367	25	5.0	0.5	0.5	8.5	10	4.5	0.2/5.2	ND	490	0	0.1	0.4
369	25	5.0	0.5	0.5	8.5	5	14.6	0/15.0	0.3/†15.3	65	0	0.1	0.3

†Final monochloramine residual assumed constant over contact time for parameter estimation.

Table 3-7. Monochloramine inactivation data for a free chlorine pretreatment level of 20 to 85 mg·min/L at pH 8.0 and 22°C

Trial No.	Reactor vol., mL	No. of oocysts in reactor, $\times 10^6$	Applied Cl_2 dose, mg/L	Final residual, mg/L	Free Cl_2 contact time, min	NH_2Cl residual post NH_4Cl addition, mg/L, Cl_2/NH_2Cl	Contact time prior to adding preformed NH_2Cl, min	Applied dose of preformed NH_2Cl, mg/L	NH_2Cl residual, mg/L, Cl_2/NH_2Cl	Final NH_2Cl residual, mg/L, Cl_2/NH_2Cl	Total contact time of NH_2Cl, min	NH_2Cl first order decay rate constant, k', min^{-1}	Net reduction in infectivity, log units	I.g.H. predicted reduction, log units
88	25	5.0	1.0	0.7	60	0.1/0.4	30	1.6	0/1.9	0/1.9	240	2.0×10^{-4}	1.0	0.4
124	25	5.0	0.7	0	60	NA	NA	6.7	0.2/6.7	0/6.2	480	1.8×10^{-4}	0.7	1.2
132	25	5.0	1.4	0.7	60	0/0.8	30	5.9	0.2/6.1	0.2/5.9	510	8.0×10^{-5}	< 0.8	1.5
155	25	5.0	0.9	0.6	30	0/0.8	NA	0	NA	0/0.8	96	2.6×10^{-4}	< 0.1	0.1
161	25	3.0	0.4	0	45	NA	NA	3.1	0/3.0	0/2.8	240	1.7×10^{-4}	1.2	0.5
192	25	5.0	1.5	1.1	48	0/1.2	NA	0	NA	0/1.2	254	0	1.1	0.3
275	25	5.0	1.0	0.8	52	0/1.0	NA	0	NA	0/0.8	240	7.1×10^{-4}	< 0.3	0.2
509	475	7.5	2.0	1.7	30	0.1/1.7	30	0.8	0.1/2.6	0/2.2	1100	1.3×10^{-4}	< 1.3	1.1
511	475	7.5	2.0	1.7	33	0/1.7	17	2.8	0.1/4.3	0.1/†4.4	127	0	< 0.7	0.5
513	475	7.5	2.0	1.7	33	ND	0	7.3	ND	0.2/†8.5	10	0	> -0.1	0.2
636	475	15.0	2.0	1.6	30	0/1.8	NA	0	NA	0/1.8	590	6.0×10^{-5}	-0.2	0.6
638	475	15.0	2.0	1.6	30	0/1.8	14	3.0	0/4.6	0/4.4	532	6.4×10^{-5}	-0.4	1.0
649	1000	40.0	4.2	4.1	15	0/4.2	NA	0	NA	0/4.2	110	0	0.4	0.4
651	1000	40.0	4.2	4.1	15	0/4.2	NA	0	NA	0/4.2	210	4.5×10^{-5}	0.8	0.6
653	1000	40.0	4.2	4.1	15	0/4.2	NA	0	NA	0/4.1	410	7.0×10^{-5}	1.3	0.9
655	1000	40.0	4.2	4.1	15	0/4.2	NA	0	NA	0/4.0	1260	5.6×10^{-5}	2.1	1.6
659	1000	40.0	4.2	4.1	15	0.1/4.2	NA	0	NA	0/4.2	110	0	0	0.4
661	1000	40.0	4.2	4.1	15	0.1/4.2	NA	0	NA	0/4.1	200	1.2×10^{-4}	0.3	0.6
663	1000	40.0	4.2	4.1	15	0.1/4.2	NA	0	NA	0/4.2	400	9.9×10^{-5}	0.8	0.8
665	1000	40.0	4.2	4.1	15	0.1/4.2	NA	0	NA	0.1/3.9	1220	6.2×10^{-5}	1.3	1.5

Note: NA denotes not applicable.

†Final monochloramine residual assumed constant over contact time for modeling.

Table 3-8. Monochloramine inactivation data for a free chlorine pretreatment level of 725 to 2,200 mg·min/L at pH 8.0 and 22°C

Trial No.	Reactor vol., mL	No. of oocysts in reactor, $\times 10^6$	Applied Cl_2 dose, mg/L	Final residual, mg/L	Free Cl_2 contact time, min	NH_2Cl residual post NH_4Cl addition, mg/L, Cl_2/NH_2Cl	Contact time prior to addition of preformed NH_2Cl, min	Applied dose of preformed NH_2Cl, mg/L	NH_2Cl residual, mg/L, Cl_2/NH_2Cl	Final NH_2Cl residual, mg/L, Cl_2/NH_2Cl	Total contact time of NH_2Cl, min	NH_2Cl first order decay rate constant, k', min^{-1}	Net reduction in infectivity, log units	I.g.H. predicted reduction, log units
26	50	1.2	5.0	3.0	248	0/3.1	42	2.0	0.4/5.9	0/†6.4	472	0	2.3	2.4
31	50	1.0	9.2	7.9	225	0/9.2	NA	0	NA	0/8.5	495	1.5×10^{-4}	> 2.9	3.8
56	25	5.0	9.2	0.2	240	ND	30	4.6	0/9.1	ND/8.0	480	2.8×10^{-4}	> 3.2	3.7
125	25	5.0	2.9	1.9	250	0/2.2	NA	0	NA	0/2.2	265	0	0.1	0.5
154	25	5.0	2.9	2.7	280	0/2.6	NA	0	NA	0/2.5	295	1.8×10^{-4}	0.7	0.7
160	25	3.0	4.2	2.1	248	0/3.5	NA	0	NA	0/3.4	255	5.7×10^{-5}	1.3	0.9
163	25	3.0	4.2	2.3	240	0/3.5	NA	0	NA	0/†3.5	30	0	0.8	0.6
523	475	7.5	3.5	3.4	205	0/3.4	NA	0	NA	ND	10	0	-0.2	0.4

Note: NA denotes not applicable, ND not determined.

†Final monochloramine residual assumed constant over contact time for modeling.

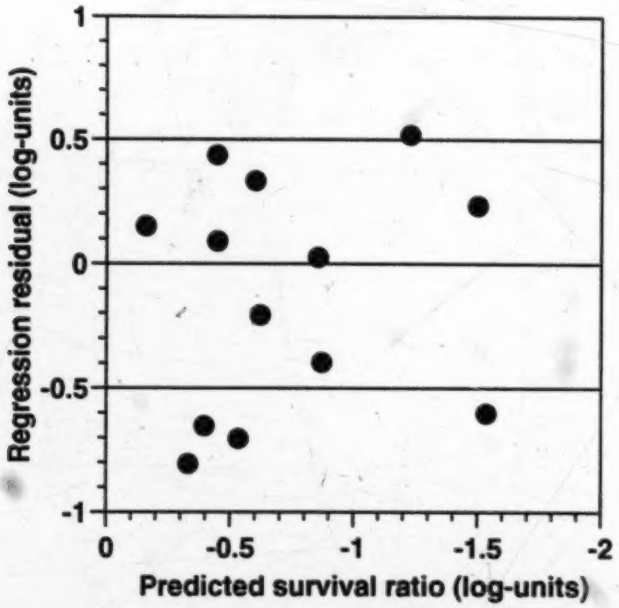

Figure 3-1. Regression residuals plotted with respect to predicted survival ratios for the monochloramine model for a free chlorine pretreatment level of 20 to 85 mg·min/L

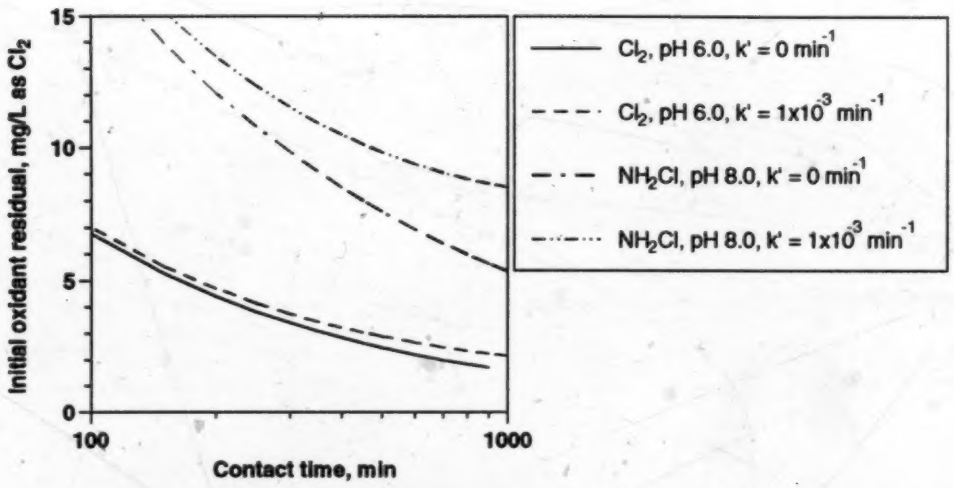

Figure 3-2. Free chlorine and preformed monochloramine process requirements for a 0.5 log-unit inactivation of *C. parvum* oocysts at 22°C for different first-order disinfectant decay rate constants

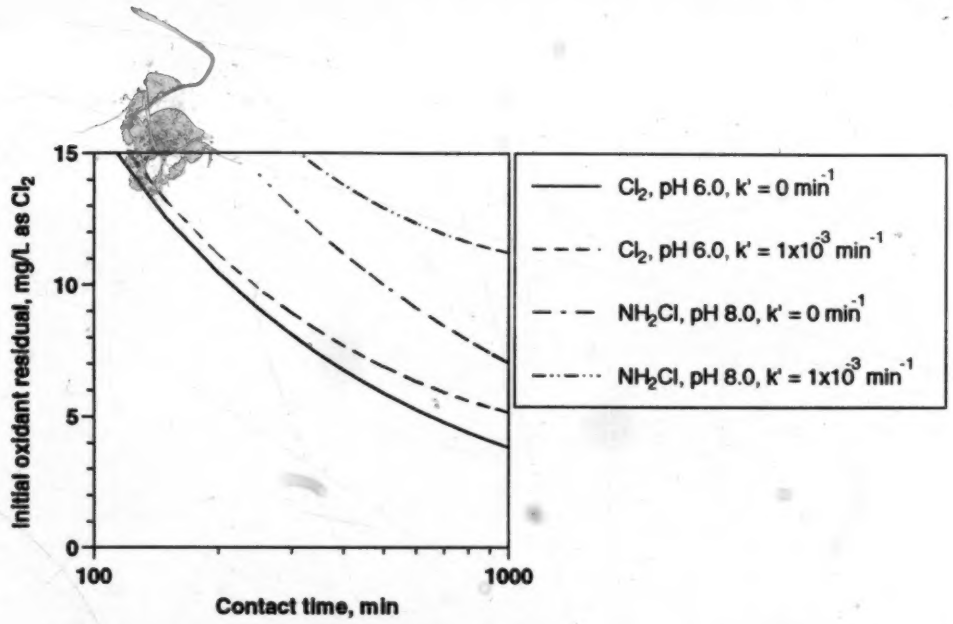

Figure 3-3. Free chlorine and preformed monochloramine process requirements for a 1.0 log-unit inactivation of *C. parvum* oocysts at 22°C for different first-order disinfectant decay rate constants

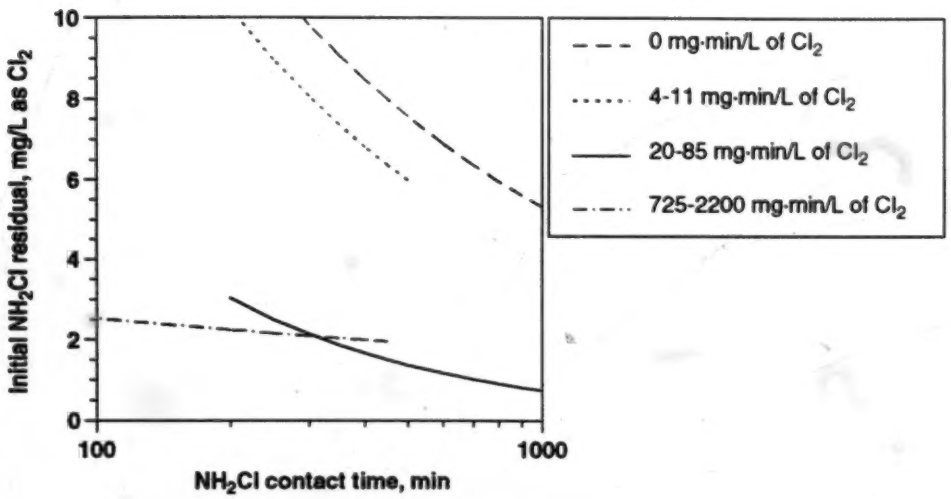

Figure 3-4. Monochloramine process requirements for a 0.5 log-unit inactivation of *C. parvum* oocysts at pH 8.0, 22°C, for various levels of free chlorine pretreatment and assuming a first-order monochloramine decay rate constant of 0 min^{-1}

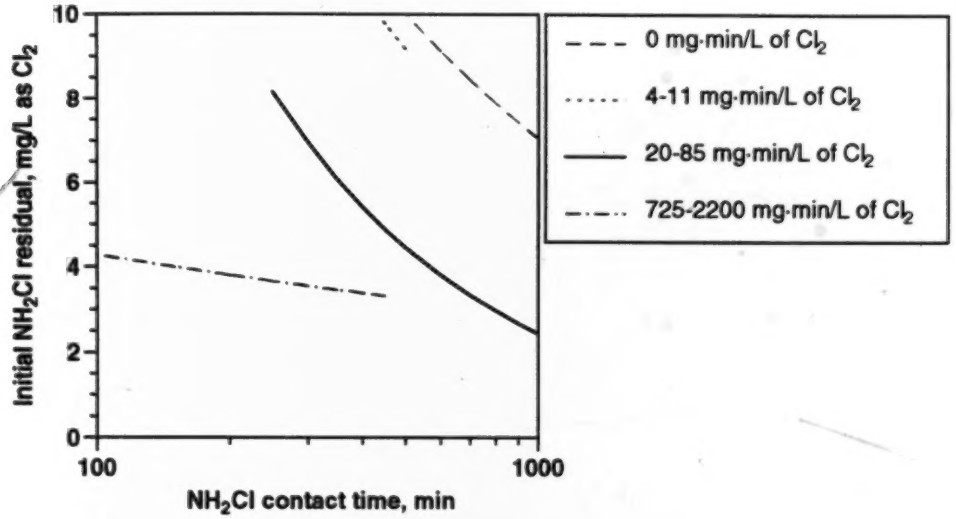

Figure 3-5. Monochloramine process requirements for a 1.0 log-unit inactivation of *C. parvum* oocysts at pH 8.0, 22°C, for various levels of free chlorine pretreatment and assuming a first-order monochloramine decay rate constant of 0 min^{-1}

Chapter 4

Kinetics of *Cryptosporidium parvum* Inactivation by Ozone[1]

4.1 Introduction

Although chlorine is most widely used to provide disinfection of potable water, the resistance of encysted protozoa to chlorine has lead to increased usage of alternative chemical disinfectants. The superior efficacy of ozone has made it one of the leading disinfectants for controlling protozoans in drinking water (Wickramanayake et al. 1985; Peeters et al. 1989; Korich et al. 1990). While there have been several studies on ozone inactivation of encysted parasites, few have used an experimental protocol that is amenable to rigorous analysis of the kinetics of inactivation and the decay of the disinfectant simultaneously. Haas and co-workers (1994a) and Finch and co-workers (1993) have published complete data sets for ozone inactivation of encysted *Giardia muris* and *C. parvum*.

Ozone disinfection data collected by Finch and co-workers (1993) involving *C. parvum* oocysts were modeled using the Incomplete gamma Hom (I.g.H.) model. The I.g.H. model can describe deviations from first-order inactivation kinetics such as tailing-off and shoulder behavior and it can account for the disappearance of chemical disinfectant (Haas and Joffe 1994b). Disinfection data sets were examined using the delete-1 jackknife to identify possible outliers. Additional experimental trials were conducted to cross-validate the Hom-type rate law describing the kinetics of *C. parvum* inactivation by ozone

[1] Gyürék, L.L, G.R. Finch, and M. Belosevic paper submitted to *Journal of Env. Eng.*, (September, 1997).

at 22°C. The use of an adequate inactivation rate law and suitable parameter estimates is essential to provide a rational basis for ozone disinfection process design.

4.2 Materials and Methods

Laboratory protocols for disinfection trials conducted in this study are the same as those used in the earlier *C. parvum* work (Finch et al. 1993). Ozone gas was generated from extra-dry oxygen using a water-cooled, corona discharge generator (Welsbach T-816, Welsbach Ozone Systems Corporation, Sunnyvale, CA). Deionized laboratory water used in the earlier work was obtained from a Milli-Q® system (OM-140, Millipore Corp., Bedford, Mass.) and from an Elga® Ultra-pure system (FisherScientific Limited, Pittsburgh, PA) for trials conducted in this study. Ozone demand-free glassware was prepared by exposing acid-washed glassware to the aqueous ozone stock solution for at least 3 hours and storage for at least 2 days at 80°C. Preparation of pH 6.0 to 8.0, 0.05 M, laboratory buffered water involved dissolution of appropriate amounts of Analar grade potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate (BDH Inc., Toronto, Ontario). Ozone demand-free water was prepared by ozonating Milli-Q® water for at least 30 min and boiling for at least 10 min. Oxford and Eppendorf pipette tips and acid-washed glassware were made ozone demand-free by either submerging and/or filling items with stock ozone solution for at least 3 h and storage in an 80°C oven for 2 days.

Ozone demand-free batch reactors used in the earlier work included 50 and 125 mL Erlenmeyer flasks. Additional trials conducted in this study used 50 mL Nalgene® (Nalge Company, Rochester, NY) polypropylene copolymer centrifugation tubes as well as 500 mL Erlenmeyer flasks and 1,000 mL borosilicate Wheaton media/lab bottles. Teflon®

(Dupont) coated magnetic stir bars were used to provide mixing in reactors. Nalgene® 50 mL centrifugation tubes were continuously agitated using a Benchtop Shaker Model G33 (New Brunswick Scientific, Edison, NJ) operated at 100 rpm. Reactor vessels were covered with aluminum foil to minimize volatilization and photodecomposition of ozone.

4.2.1 Parasite Methods

Refer to Appendix A for a detailed description of the parasite methods.

4.2.2 Disinfection Protocols

Methodologies used to conduct *C. parvum* disinfection trials in the present study are similar to those used in the earlier work (Finch et al. 1993). *C. parvum* oocysts were suspended in ozone demand-free, 0.05 M, phosphate buffer to give a concentration ranging from 4.0×10^3 to 7.2×10^5 oocysts per mL. To prepare an aqueous ozone stock solution having an approximate concentration of 20 mg/L, an ozone-oxygen gas mixture was bubbled through 400 mL of Milli-Q® water in two 500 mL gas absorption flasks connected in series for at least 20 min. The concentration of stock aqueous ozone was measured using ultraviolet spectrophotometry at 260 nm and a molar absorption coefficient of 3,300 $M^{-1}cm^{-1}$ (Hart et al. 1983). A 1.0 cm quartz cuvette and model 601 Milton Roy spectrophotometer were used to take stock ozone measurements three times prior to and twice following addition of the calibrated volume of stock ozone solution to the reactor. The mean of these five stock ozone measurements was used to calculate the ozone dose applied to the reactor using a mass-calibrated Oxford pipette. A diode-array spectrophotometer (Hewlett-Packard model 8452A) monitored the absorbance at 260 nm of the reactor contents. The reactor was continuously sampled in a closed loop at a flow

rate of 8 mL/min. The sample was carried through a short piece of small diameter Teflon tubing to a 35 μL flow cell with a light path of 1.0 cm. Hoigné and Bader (1994) have suggested the direct UV method is acceptable for waters of low background absorbance, i.e. $A_{258} \leq 0.01$ cm^{-1}, provided the ozone dose is ≥0.5 mg/L. Any ozone remaining at the end of the (predetermined) contact time was neutralized using 1M sodium formate (BDH Inc., AnalaR grade). The mass of sodium formate added did not interfere with absorbance measurements and had no effect on *C. parvum* infectivity. Positive controls were used to account for any potential effect of sodium formate and oocyst recovery procedure.

The difference between the final baseline absorbance (following sodium formate addition) and measured absorbances over the contact time, multiplied by a concentration factor of 14.55, was used to calculate ozone residuals. The applied ozone dose is the mass of ozone delivered from the stock solution divided by the final volume of the solution in the reactor. The initial ozone residual is the ozone concentration measured in the reactor immediately following addition of the stock solution. The instantaneous ozone demand is the difference between the calculated applied ozone dose and the measured initial ozone residual.

4.2.3 Interpretation of Infectivity Data

The viability of *C. parvum* oocysts following exposure to ozone was determined using animal infectivity and a logistic dose-response model. The survival ratio, expressed in log-units, was estimated from:

$$\log \frac{N}{N_o} = \log\left(\frac{n}{n_o}\right) \qquad (3\text{-}2)$$

where n is the estimated infectious dose per animal after disinfection and n_o is the number of oocysts given to each animal. The estimated infectious dose was calculated from the logit mean response given by (Neter et al. 1989):

$$\pi' = \ln\frac{P}{1-P} = \beta_o + \beta_1 X \qquad (3\text{-}3)$$

where P is the proportion of animals in the cohort scoring positive for a specified inoculum X (log-units) and β are the logit response model parameters. Model parameters were estimated using the method of maximum likelihood (ML) and Microsoft® Excel® 5.0. ML estimators of β_o and β_1 are those values that maximize the $\log_e$ of the likelihood function, L, (Brand et al. 1973; Cox 1970):

$$\ln L = \sum_{i=1}^{a} Y_i(\beta_o + \beta_1 X_i) - \sum_{i=1}^{a} \ln\left[1 + \exp(\beta_o + \beta_1 X_i)\right] \qquad (3\text{-}4)$$

where $Y_i = 0, 1$; and $i = 1, 2, \ldots$, up to a, the number of neonate CD-1 mice used in the dose-response study for a particular batch of oocysts.

Logistic dose-response models for *C. parvum* in CD-1 mice were developed for different batches of oocysts collected and used in the experiments. In the previously published *C. parvum* study (trials 16-107), maximum likelihood estimates of β_o and β_1 were -6.74 and 3.55, respectively (Finch et al. 1993). The dose-response data on which these logit parameters were estimated was evaluated and the scores of 23 mice (out of 224), comprising 3 cohorts, were considered to be outliers. The observed fraction of the these cohorts scoring positive for infection differed by >0.5 from the expectation based on

all dose-response data for that particular batch of oocysts. Omitting these 3 outlier cohorts, the logit parameters were estimated to be -7.96 and 4.06, respectively.

4.2.4 Kinetic Inactivation Data Analysis

Kinetic models have been used to describe the various survival curves observed for organisms exposed to chemical disinfectants as a function of disinfectant concentration and contact time. Laboratory data are typically interpreted using Chick-Watson kinetics and CT values calculated to design contactor systems for a given level of organism inactivation (Hoff 1987). The Chick-Watson pseudo first-order rate law (equation 2-2) was integrated in Chapter 2 to give the Chick-Watson model:

$$\log \frac{N}{N_o} = -kC^n T \qquad (2\text{-}3)$$

where k is an inactivation rate constant; C is the concentration of the chemical disinfectant assumed to remain approximately constant, mg/L; n is an empirical constant; and T is the total applied contact time, min. To simplify contactor system design and regulatory practice, the value of n is generally assumed to be equal to unity.

The assumption of a constant disinfectant concentration is often not appropriate. Immediately following addition of a chemical disinfectant, a fraction of the oxidant is consumed by an instantaneous demand. This fast reaction is followed by a slower rate of consumption. First-order kinetics have been found to adequately describe the decomposition of ozone in aqueous solution (Hoigné and Bader 1994; Masschelein 1982):

$$C = C_o \exp(-k't) \qquad (2\text{-}14)$$

where C and C_o are the disinfectant residual, mg/L, at time t and time zero respectively, and k′ is the first-order decay rate constant, min^{-1}. The ozone decay rate constants, k', for each trial were calculated using the Solver function of Microsoft® Excel® to regress equation 2-14 using the method of least-squares and a minimum of five data points from the ozone time-concentration profile including the initial and final measured ozone residual. Substitution of equation 2-14 into the Chick-Watson differential rate law and integration gives a Chick-Watson (C-W) model that accounts for first-order ozone decomposition:

$$\log\frac{N}{N_o} = -\frac{k}{k'n}(C_o^n - C_f^n) \qquad (2\text{-}16)$$

where C_o and C_f are measured ozone residuals, expressed as mg/L, at the beginning and end of the applied contact time, respectively.

Exponential inactivation kinetics predicted by the Chick-Watson model are rarely observed in laboratory studies conducted in batch reactors under disinfectant demand-free conditions (Fair et al. 1948; Hiatt 1964; Hoff 1986; Hom 1972). A Hom-type differential rate law that describe deviations from exponential die-off kinetics and account for first-order disinfectant decomposition is given by:

$$\frac{dN}{dt} = -km NC_o^n e^{-k'tn} t^{m-1} \qquad (2\text{-}15)$$

where m is an empirical constant. This expression can be integrated to give the robust Incomplete gamma Hom (I.g.H.) model (Haas and Joffe 1994b):

$$\log\frac{N}{N_o} = \frac{-mkC_o^n}{(nk')^m}\gamma(m, nk'T) \qquad m > 0,\ nk'T \geq 0 \qquad (2\text{-}19)$$

where $\gamma(\alpha, x)$ is the Incomplete gamma function that requires $\alpha > 0$ and $x \geq 0$ (Deming 1944).

For reactors in which instantaneous ozone demand is a significant fraction of the applied ozone dose, the fitted Hom-type rate law (equation 2-15) can be used to calculate the amount of *C. parvum* inactivation that can be attributed to the instantaneous ozone consumed. First-order ozone decomposition in a reactor can be conceptualized as occurring in two stages: an initial fast rate lasting 10 seconds with the ozone decay rate constant given by $\ln[C_o/\text{applied dose}]/0.17\ \text{min}^{-1}$, followed by a slower rate of ozone decay. The bulstoer function of Mathcad Plus 6.0 can be used to calculate predicted levels of inactivation by simultaneously solving equation 2-15 and an ozone concentration profile defined by two different first-order rate constants for contact times less than and greater than 10 s.

4.2.5 Model-Data Set Behavior

A maximum likelihood approach was used to estimate I.g.H. model parameters as described in Chapter 3. The Minerr function of Mathcad Plus 6.0 (MathSoft, Inc., Cambridge, MA) was used to maximize the logarithm of the likelihood function, lnL. The Mathcad solution was found to be sensitive to initial parameter guess values. Different sets of initial values were therefore used to confirm global maxima. Parameter estimate joint confidence regions can be constructed for a nominal confidence level using the likelihood ratio test (Beale 1960; Lampton et al. 1976). A nominal $1-\alpha$ profile likelihood for maximum likelihood estimators is given by all values of $\beta = (k, m, n)'$ such that (Seber and Wild 1989):

$$\ln L_{1-\alpha}(\beta) \geq \ln L_{max}(\hat{\beta}) - \frac{1}{2}\chi^2_{p,\alpha} \qquad (3\text{-}10)$$

where $\chi^2_{p,\alpha}$ is the (1-α)% percentile of a chi-squared distribution with p degrees of freedom; $L(\beta)$ denotes the likelihood function; and $L_{max}(\hat{\beta})$ its supremum. Upper and lower 95% marginal confidence limits were also computed for each of the I.g.H. parameter estimates using the likelihood ratio test. Mathcad Plus 6.0 was used to compute limits by varying one parameter at a time with the other two parameters fixed to their optimal estimates such that the equality constraint was satisfied.

4.2.6 Jackknife Resampling

A computationally intensive resampling method, the delete-1 jackknife, was applied to data collected in the earlier studies by Finch and co-workers (1993) to identify individual trials of high leverage (possible outliers) with respect to I.g.H. model. Duncan (1978) discussed the application of the jackknife method for calculating parameter estimates and confidence regions for the parameters of a nonlinear regression model. The delete-1 jackknife requires the fitting of v+1 nonlinear regressions, where v is the number of trials in the data set. Stating $\hat{\beta}_i$ to be the estimate of β when the i^{th} trial is deleted from the data set, pseudo-values are defined as (Duncan 1978):

$$P_i = v\hat{\beta} - (v-1)\hat{\beta}_i \qquad (4\text{-}1)$$

Jackknife parameter estimates are an average of the pseudo-values, namely:

$$\bar{\beta}_J = \frac{1}{v}\sum_{i=1}^{v} P_i \qquad (4\text{-}2)$$

Rather than use the pseudo-values (data not shown) to examine the influence of individual trials on I.g.H. parameter estimates, high leverage trials were identified using likelihood distances (Cook and Wang 1983):

$$LD_i = 2\left(\ln L_{max}(\hat{\beta}) - \ln L_{max}(\hat{\beta}_i)\right) \qquad (4\text{-}3)$$

where LD_i is the likelihood distance when the i^{th} trial is deleted from a data set.

Model parameters were not estimated using the delete-1 jackknife procedure. Jackknife parameter estimates can be free of error distribution assumptions in nonlinear models with small to medium-size samples and may be reasonable under heterogeneity of error variance (Fox et al. 1980; Simonoff and Tsai 1986). The delete-1 jackknife procedure, in the usual form described by equations 4-1 and 4-2, applied to an unbalanced regression data set can lead to unstable jackknife estimates (Hinkley 1983). A weighted jackknife, with weights proportional to the determinant of the Fisher information matrix, has been proposed to account for the unbalanced nature of regression data (Wu 1986). Application of a weighted delete-1 jackknife to estimate parameters for the I.g.H. model at 22°C may warrant further study.

4.2.7 Arrhenius Analysis

An empirical relationship that often describes the dependence of the rate constant on temperature is the classic Arrhenius equation (Levenspiel 1962):

$$k = A \cdot \exp\left[-\frac{E_a}{RT}\right] \qquad (4\text{-}4)$$

where A is the pre-exponential factor or frequency factor, E_a is the activation energy, R is the universal gas constant 1.987 cal/g-mole K, and T is the temperature in Kelvin. Use of

equation 4-4 results in highly correlated estimates when the range of observed temperatures is small relative to the mean temperature (Bates and Watts 1988). To reduce correlation, the activation energy and pre-exponential factor are estimated by centering temperatures about an intermediate temperature, T_o:

$$k_T = A \cdot \exp\left[-\frac{E_a}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_o}\right)\right] \qquad (4\text{-}5)$$

To calculate the activation energy, it is necessary that the inactivation rate constant refer to the same rate law. In the case of the I.g.H. model, m and n must be the same for models at different temperatures.

4.3 Results and Discussion

Disinfection data published by Finch et al. (1993) was used to estimate parameters of the I.g.H. model describing ozone inactivation of *C. parvum* oocysts. The original experimental data (trials 16 to 107) are summarized in Tables 4-2 and 4-4 for approximate temperatures of 7° and 22°C, respectively. A summary of the neonatal CD-1 mice infectivity data for these ozone disinfection trials is given in Tables 4-3 and 4-5. I.g.H. model parameter estimates for the 7° and 22°C data are summarized in Table 4-6.

The agreement between observed inactivation ratios and I.g.H. model predicted ratios listed in Table 4-2 is indicative of good model fit at 7°C. The square root of the maximum likelihood regression error variance (σ) was estimated to be 0.43. Marginal 95% (approximate) confidence limits for parameter estimates of the I.g.H. model at 7°C are provided in Table 4-6. Comparing the spread of the marginal confidence for m and n, there is less certainty regarding the influence of ozone concentration on the rate of *C.*

parvum inactivation by ozone compared to that of contact time. Instability in the parameter estimates of nonlinear regression models can compensate however so as not to cause instability in the response function (Box and Lucas 1959). This is the case for the I.g.H. model at 7°C since the model fitted survival ratios agree satisfactorily with the observed survival ratios as shown in Table 4-2.

A good model fit is not provided by the I.g.H. model at 22°C (including trial 30) as indicated by an estimated σ value of 0.79. The marginal 95% confidence limit for n extends from <0 to 0.98. The Incomplete gamma function is ill-defined for any negative value of n. To identify trials of high leverage which may be possible outliers leading to inadequate model fit, the delete-1 jackknife was applied to the *C. parvum* data and likelihood distances calculated. An index plot of the absolute values of likelihood distances is illustrated in Figure 4-1. The sensitivity of the likelihood function to trial 30 is indicative of an outlier response. Omission of trial 30 from the data set lead to significant improvement in model fit as indicated by an estimated σ value of 0.55. Model fitted inactivation ratios given in Table 4-4 were calculated based on the omission of trial 30. The general agreement between observed inactivation ratios and model fitted ratios as shown in Table 4-4 indicates that the model response function is stable and reliable. The instability or uncertainty associated with n does not invalidate the I.g.H. model at 22°C. As previously mentioned, instability in the parameter estimates can compensate so as not to cause instability in the response function (Box and Lucas 1959). The validity of the I.g.H. model at 22°C was further tested by conducting additional disinfection trials and comparing observed inactivation ratios to model predicted ratios.

4.3.1 Model Assumptions

The method of maximum likelihood is restrictive in requiring explicit assumptions concerning the form of the probability distribution of the regression errors. This means that two mathematical models are being considered simultaneously: one describing the kinetic reacting system and the other a probability distribution for the errors (Reilly and Blau 1974). Errors in the regression model were assumed normally distributed, independent, with zero mean and a constant error variance, σ^2. Model inadequacies can often be detected through an analysis of the residuals of a model (Box and Hunter 1965), provided instrinsic curvature is negligible for nonlinear models (Seber and Wild 1989).

For the I.g.H. models at 7° and 22°C, regression residuals (observed - predicted survival ratios) were plotted against all variables, i.e. predicted and observed survival ratios, applied contact time, and initial measured ozone residual. Residual plots indicated disturbances are randomly distributed, independent of all the variables, with approximate zero means and constant variance. These figures (not shown) are similar in appearance to Figure 4-3 which is a plot of residuals with respect to predicted survival ratios for *C. parvum* at 7° and 22°C. Trials with observed survival ratios below infectivity detection limits are not shown in Figure 4-3. A Kolmogorov-Smirnov goodness of fit test was used to examine the normality of residuals for the I.g.H. models (Haas and Jacangelo 1993). Maximum absolute differences (D) between the calculated Kaplan-Meir product-limit cumulative distribution function and standard normal distribution were calculated and are provided in Table 4-6. These values were compared with critical D values tabulated elsewhere (Lilliefors 1967). Calculated D values were significant for $\alpha > 0.20$. The

probability of erroneously rejecting the assumption of normality is >0.20 which suggests that errors are consistent with a normal distribution and that maximum likelihood assumptions are satisfied.

4.3.2 Nonlinearity of Model-Data Set Behavior

Modeling of microbial inactivation kinetics requires not only an adequate (nonlinear) model but also appropriate model-data set behavior. Because most statistical procedures are derived from analogies with linear models (with respect to the parameters) and normality, it is preferable that nonlinear model-data set behavior approach linearity (Atkinson and Hunter 1968). Model-data set behavior is linear if the expectation surface of the likelihood function is sufficiently flat to be replaced by a tangent plane (planar assumption), and if straight, parallel equispaced lines in the parameter space map into nearly straight, parallel equispaced lines on the expectation surface (uniform coordinate assumption) (Bates and Watts 1988). The planar and uniform coordinate assumptions are related to intrinsic nonlinearity and parameter-effects curvature, respectively. Profile likelihoods constructed using equation 3-10 are invariant to parameter-effects curvature and can thus be used to assess the uniform coordinate assumption. Although Bates-Watts parameter-effects curvature arrays can be computed to identify model-data set nonlinearity (Bates and Watts 1988), a graphical approach is generally more informative (Cook 1986).

The profile likelihood for the m-n parameter subset for the I.g.H. model at 7° and 22°C, conditional for optimal $\hat{k}$, for an approximate 95% confidence level is illustrated in Figure 4-2. Transform (Fortner Research Inc.) was used to interpolate contours. The profile likelihoods are ellipsoidal, and not banana shaped, which is indicative of a well-

conditioned likelihood surface and negligible parameter-effects curvature. Hence the uniform coordinate assumption is satisfied. Profile likelihoods for the other parameter subsets (not shown) were similar in appearance. I.g.H. models at 7° and 22°C were not tested for intrinsic nonlinearity because the planar assumption is generally satisfied as demonstrated by an analysis of 67 real (not simulated) model-data set combinations by Bates and Watts (1988).

4.3.3 Cross-Validation of I.g.H. Model at 22°C

Additional trials, denoted by an 'a' in Table 4-4, were conducted at pH values ranging from 6.0 to 8.0 to cross-validate the I.g.H. model at 22°C. The measured ozone residuals used to calculate the ozone decay rate constants listed in Table 4-4 for the cross-validation trials are summarized in Appendix C. The use of 50 mL Nalgene® reactors resulted in relatively high oocyst recoveries but also greater variation in the instantaneous ozone demand and rate of ozone disappearance compared with the larger reactor volumes used in the earlier work. For trials a83 and a461, instantaneous ozone demand was greater than 10% of the applied dose. For these two trials the contribution of instantaneous ozone demand on the model fitted survival ratio was calculated as previously described and is denoted by a '†' in Table 4-4. *C. parvum* oocyst inactivation that could be attributed to instantaneous ozone demand (≤0.7 mg/L) was found to be negligible, i.e., <0.1 log-units. This emphasizes the need to define disinfection efficiency on the basis of measured disinfectant residuals in reactors and not the dose.

Differences between observed and predicted levels of inactivation for cross-validation trials are plotted, with respect to predicted values, in Figure 4-2. Predicted

inactivation levels lie within 0.6 log-units of observed reductions in infectivity for levels of inactivation ranging from 1 to 4 log-units. This 0.6 log-unit difference can be attributed to the inherent variability associated with the neonate CD-1 mice dose-response model. The I.g.H. model at 22°C was able to reliably predict inactivation ratios in phosphate buffered laboratory water. The marginal 95% confidence limit for n was however estimated to range from 0.16 to 1.09, as listed in Table 4-6. It is recommended that the cross-validation data set be augmented with a few additional ozone disinfection trials such that an independent set of I.g.H. parameters can be estimated. The estimation of an independent set of I.g.H. model parameter estimates for 22°C would permit a comparison to the present model.

The I.g.H. model appears able to account for first-order ozone decay rates ranging from 0.1 to 1.7 min^{-1} in phosphate buffered laboratory water. The general agreement between observed and predicted results for cross-validation trials suggests that the resistance of *C. parvum* oocysts to ozone at pH 6.0 and 8.0 differs by <0.6 log-units compared with pH 7.0 at 22°C. This suggests that the permeability of the oocyst wall to aqueous ozone is likely to be the same within the pH range of 6.0 to 8.0.

4.3.4 Effect of Temperature

The thermodynamic properties of ozone inactivation of *C. parvum* oocysts was evaluated using the 7° and 22°C data. An empirical relationship which often describes the dependence of the rate constant on temperature is the classic Arrhenius expression (equation 4-4). To calculate the activation energy, it was necessary to assume the same m and n parameters for the 7° and 22°C Hom rate laws. Based on the profile likelihood

illustrated in Figure 4-2 for the m-n parameter subset, a value of 0.67 and 0.73 was considered appropriate for m and n, respectively. Based on these parameter values, the k parameter was re-estimated and found to be 0.54 and 0.76 for 7° and 22°C, respectively. Details regarding the Arrhenius I.g.H. models are given in Table 4-6. The activation energy and pre-exponential factor were calculated to be 3.8 kcal/mol and 0.64 min^{-1}, respectively. In comparison, activation energies of 40 to 80 and 3 to 7 kcal/mol are considered characteristic of protein denaturation and the breaking hydrogen bonds, respectively (Prokop and Humphrey 1970).

The rate of *C. parvum* inactivation by ozone was found to increase with increasing temperature. An increase in temperature will increase the rate of chemical reactions and may also affect the rate of ozone diffusion across the oocyst wall. Chemical reaction rates are more susceptible to changes in temperature than the rates of reaction controlled by physical processes such as diffusion (Roy et al. 1981). In general a 10°C rise in temperature is considered to double the reaction rate constant. This corresponds to an activation energy of 12 kcal/mol which is the median of range of most reactions in solution (James M. Montgomery Consulting Engineers 1985). A useful technique to measure the effect of temperature on reaction rates is given by (Russell 1992):

$$\theta^{(T_2-T_1)} = \frac{k_2}{k_1} \qquad (4\text{-}6)$$

in which k_2 and k_1 are the rate constants at temperatures T_2 and T_1, respectively. For the I.g.H. model inactivation rate constants at 7° and 22°C, the temperature coefficient, θ^1, for *C. parvum* inactivation by ozone is 1.023. It is more common to specify the θ^{10}, or

Q_{10} value, which is the change in activity per 10°C rise in temperature. The Q_{10} was calculated to be 1.26 which indicates that the general rule of the reaction rate constant doubling for a 10°C rise in temperature does not apply to *C. parvum* inactivation by ozone.

The magnitude of the inactivation energy provides insight into whether the process of *C. parvum* inactivation by ozone is limited by the mass transfer rate or the chemical reaction rate between the ozone and the oocyst. The magnitude of the activation energy, 3.8 kcal/mol, suggests that inactivation of *C. parvum* oocysts by ozone is controlled entirely by the mass transfer of ozone into the oocyst. A similar finding was reported by Roy et al. (1981) for the inactivation of poliovirus 1 by ozone at pH 7.2 with an estimated activation energy of 3.6 kcal/mol. Activation energies for ozone inactivation of *G. muris*, *G. lamblia* and *Naegleria gruberi* cysts at pH 7.0 were reported to be 9.7, 16.7, and 7.5 kcal/mol, respectively, based on a semi-batch methodology (Wickramanayake et al. 1985; Wickramanayake and Sproul 1988). For these encysted protozoa the magnitude of activation energy, 7.5 to 16.7 kcal/mol, suggests that the rate limiting step may be controlled by a combination of mass transfer and chemical reaction rates.

4.3.5 Application of Kinetic Model

For a given first-order decay rate constant at a given temperature, the initial ozone concentration and contact time required for a specified level of inactivation (in an ideal plug flow reactor) can be calculated on the basis of I.g.H. models. Kinetic modeling is based on the assumption that the first-order disinfectant decay rate constant is independent of the organisms present and is only a function of the water characteristics and disinfectant

(Anmangandla 1993); conversely, model parameters k, m, and n, are independent of the water characteristics and only a function of the disinfectant and organism. The decay rate constant relates the ozone concentration at time t to the measured initial residual immediately following addition of the stock ozone solution to the reactor and not to the applied ozone dose. This is directly applicable to a second stage (ideal) plug-flow reactor that is preceded by a completely mixed contactor providing rapid mass transfer of ozone and satisfying any instantaneous ozone demands.

The initial ozone residual and contact time required for 2.0 log-units *C. parvum* inactivation at 7° and 22°C is illustrated in Figure 4-4 based on the I.g.H. model for first-order ozone decay rate constants of 0 and 0.2 min^{-1}. I.g.H. parameters m and n were estimated to have nearly the same value, less than unity, which means that *C. parvum* inactivation behavior is characterized by tailing-off and that ozone concentration and contact time are of similar importance on inactivation kinetics. Previous studies have reported that ozone concentration is the predominant factor which suggested that contactors should be designed to maximize the ozone residual for a given ozone dosage for shorter applied contact times (Labatiuk et al. 1992; Zhou and Smith 1994). The I.g.H. model supports this design principle for contactor systems in which aqueous ozone residuals rapidly decompose (assuming that any instantaneous ozone demand has already been satisfied).

For a negligible first-order ozone decay rate constant at 7°C, the model predicts an ozone residual of 0.5 mg/L applied for 15 min and 2.5 mg/L applied for 3 min gives 1.9 and 2.2 log-units inactivation, respectively. For a first-order decay rate constant of 0.2

min^{-1}, predicted levels of inactivation decrease to 0.9 and 1.8 log-units, respectively. The effect of higher ozone decay rates on levels of organism inactivation is less significant for shorter contact times because there is less time available for ozone decomposition. The rate at which ozone decays is a function of water quality parameters such as pH, dissolved organic carbon content, alkalinity, and temperature. Figure 4-4 suggests that ozone efficacy is greater at 22°C compared with 7°C given a similar ozone decay rate coefficient. In addition, an increase in the ozone decay rate constant reduces the expected level of inactivation to a much greater extent at lower temperatures. Under certain conditions such as longer contact times, as illustrated in Figure 4-4, ozone efficacy can be similar at 7° and 22°C.

The SWTR Guidance Manual provides CT tables for several disinfectants which specify the level of inactivation credited based on calculated CT values (USEPA 1990). The SWTR currently requires 3 log-units removal-inactivation of *Giardia* cysts and assumes effective filtration at a conventional treatment plant provides 2.5 log-units removal. Based on the ozone disinfection data of Korich et al. (1990) involving *C. parvum* oocysts, a CT value of 3.75 mg·min/L was proposed for crediting 2 log-units inactivation at 25°C and pH 7.0 (Black and Veatch 1994). Korich et al. (1990) used a semi-batch methodology, which involves continuous addition of oxygen-ozone gas to the reactor, to ozonate 10^8 oocysts suspended in 350 mL of 0.01 M phosphate buffer. A constant ozone residual of 1.0 mg/L was applied for 5 min to effect 2 log-units inactivation based on neonatal BALB/c mice infectivity data. Perrine et al. (1990) used a semi-batch methodology to maintain a constant ozone residual of 0.44 mg/L for 6 min to

effect 2 log-units inactivation *C. parvum* corresponding to a CT of 2.6 mg·min/L. As shown in Figure 4-4, ozone design requirements based on a CT value of 3.75 mg·min/L closely approximate I.g.H. modeled requirements under ozone demand-free conditions, $k'=0$ min^{-1}. Parameter estimates of m and n were nearly the same which explains the (apparent) applicability of the CT concept.

The CT concept stems from the Chick-Watson model (equation 2-3) which is derived on the basis of a constant disinfectant residual. Disinfectant demand-free conditions are unlikely for most oxidants, in particular ozone, and natural waters (Hoff 1987), resulting in the disinfectant concentration being less in the effluent compared to other parts of a reactor (Lawler and Singer 1993). The SWTR therefore specifies that the characteristic disinfectant concentration be the effluent concentration from the reactor. For ozone demand conditions, the proposed CT design criteria of 3.75 mg·min/L therefore becomes a C_fT criteria in which C_f is the effluent ozone residual. The initial ozone residual and contact time required based on this SWTR approach are plotted in Figure 4-4 for an ozone decay rate constant of 0.2 min^{-1}. The level of inactivation is underestimated in specifying the effluent ozone residual be considered a constant residual over the entire contact time and hence the SWTR approach leads to significant facility over design.

Lawler and Singer (1993) similarly demonstrated the potential for over design of chlorine disinfection systems and recommended that a first-order disinfectant decay rate constant be included in the Chick-Watson (C-W) model (equation 2-16). Parameter estimates for this C-W model, with n fixed at unity, are presented in Table 4-6. The quality of the C-W model fit is poorer than that given by the I.g.H. model. The C-W

model underestimates the level of *C. parvum* inactivation compared to the I.g.H. model as illustrated in Figure 4-4, the extent of which increases as the ozone decay rate increases. This difference is caused by the tailing-off of the survival curve for *C. parvum* oocysts exposed to ozone (the case where $\bar{m} < 1$). This underscores the inability of the Chick-Watson inactivation rate law (equation 2-2) to describe the tailing-off effect.

4.4 Conclusions

An adequate inactivation rate law and suitable parameter estimates are essential to provide a rational basis for the design of ozone disinfection systems for controlling *C. parvum*. The experimental data of Finch et al. (1993) was analyzed using the robust (nonlinear) Incomplete gamma Hom (I.g.H.) model that accounts for disinfectant decomposition. A delete-1 jackknife resampling procedure was used to identify possible outliers in the data. Profile likelihoods of I.g.H. model parameter estimates demonstrated negligible parameter-effects curvature and hence linear model-data set behavior at 7° and 22°C.

I.g.H. parameters m and n were estimated to have nearly the same value, less than unity, which means that *C. parvum* inactivation behavior is characterized by tailing-off and ozone concentration and contact time are of similar importance to inactivation kinetics. The fitted Hom-type rate law at 22°C was cross-validated using additional trials conducted in this study following methods used in the earlier work of Finch et al. (1993). Observed and predicted cross-validation results demonstrated that the resistance of *C. parvum* oocysts to ozone is not appreciably different (<0.6 log-units) between pH 6.0 to 8.0. This suggests that the permeability of the oocyst wall to ozone is the same within the

pH range of 6.0 to 8.0. It is recommended that the cross-validation data set be augmented with a few additional trials to permit estimation of an independent set of I.g.H. model parameter estimates for comparison to the present model at 22°C.

The rate of *C. parvum* inactivation by ozone was found to increase with increasing temperatures with all of the other environmental variables the same. The Arrhenius activation energy was calculated to be 3.8 kcal/mol which suggests that inactivation of *C. parvum* by ozone is entirely controlled by mass transfer of ozone into the oocyst. The general rule that the reaction rate doubles for a 10°C rise in temperature does not apply to ozone inactivation of *C. parvum* because the Q_{10} value is 1.26.

I.g.H. models were used to develop process design requirements for 2 log-units inactivation of *C. parvum* by ozone at 7° and 22°C. For natural water contactor systems in which aqueous ozone residuals disappear rapidly, I.g.H. modeling confirms the design concept of applying higher ozone residuals for shorter contact times to maximize inactivation levels of *C. parvum*. Disinfection design requirements formulated on the basis on I.g.H. models are superior to the design criteria based on the current SWTR CT approach and a Chick-Watson model that accounts for disinfectant decay. The I.g.H. model offers a rational basis for designing ozone contactor systems to reliably meet *C. parvum* inactivation requirements.

4.5 References

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Table 4-1. Logit response parameter estimates for the neonate CD-1 animal model for disinfection trials corresponding to different batches of *C. parvum* oocysts

Trial no.	$\hat{\beta}_o$	$\hat{\beta}_1$	+/- 90% $\hat{\beta}_o$ Limits	+/- 90% $\hat{\beta}_1$ Limits
16-107	-7.96	4.06	-7.6, -8.3	4.2, 3.9
a83	-4.42	2.44	-4.0, -4.8	2.7, 2.2
a210, a211	-3.52	1.88	-3.2, -3.9	2.1, 1.7
a221	-5.55	2.59	-5.1, -6.0	2.8, 2.4
a414, a461	-6.02	2.37	-5.7, -6.4	2.5, 2.2
a672	-5.64	3.17	NC	NC
a859	-7.79	4.41	NC	NC

Note: 'a' is used to distinguish the experimental trials conducted in this work from data previously collected by Finch and co-workers (1993). NC - Not Calculated

Table 4-2. Ozone inactivation data of *C. parvum* oocysts suspended in phosphate buffer, 0.05 M, at 7°C

Trial No.	pH	Temp., °C	Reactor volume, mL	No. of oocysts in reactor	Applied ozone dose, mg/L	Initial ozone residual, mg/L	Final ozone residual, mg/L	Contact time, min	First-order ozone decay rate, min^{-1}	Net reduction in infectivity, log-units	I.g.H. predicted reduction, log-units
62	6.9	7	50	1.0×10^6	1.6	1.5	0.9	4.9	0.11	1.5	1.8
64	6.9	7	50	1.0×10^6	1.5	1.4	0.5	10.3	0.11	> 1.3	2.4
68	6.9	5	50	1.1×10^6	2.9	2.6	1.2	5.0	0.16	2.7	2.5
70	6.9	5	50	1.1×10^6	2.0	1.9	0.3	9.8	0.17	3.0	2.5
73	6.9	3	50	1.1×10^6	0.8	0.8	0.4	4.8	0.12	0.9	1.0
75	6.9	3	50	1.2×10^6	0.6	0.6	0.2	9.9	0.13	0.6	1.1
78	7.0	8	50	1.2×10^6	2.4	2.3	0.9	9.8	0.09	> 3.9	3.7
80	7.0	8	50	1.2×10^6	1.3	1.3	0.8	5.0	0.11	> 1.0	1.6
84	7.0	7	50	1.0×10^6	2.3	2.3	0.7	9.9	0.12	3.3	3.3
88	7.0	10	50	1.2×10^6	2.6	2.6	1.3	8.8	0.08	3.1	3.9
93	7.0	8	50	1.1×10^6	1.9	1.9	0.5	14.8	0.10	3.6	3.5

Source: Finch et al. (1993)

Table 4-3. Summary of *C. parvum* oocyst infection in neonatal CD-1 mice for ozone disinfection trials at 7°C

Trial No.	Inoculum oocysts, per animal	Live animals in cohort	Infected animals in cohort	Estimated infectious dose, oocysts per animal	Reduction in infectivity, log units	Inoculum oocysts, per control animal	Live animals in control group	Infected animals in control group	Estimated infectious dose, oocysts per animal	Reduction in control infectivity, log units	Net reduction in infectivity, log-units
62	1.30×10^{3}	5	1	42	1.50	5.30×10^{1}	4	1	49	0.03	1.5
64	1.39×10^{3}	3	0	< 62	> 1.35	5.30×10^{1}	4	1	49	0.03	> 1.3
68	2.17×10^{4}	4	2	91	2.38	8.10×10^{1}	8	6	170	-0.32	2.7
70	2.19×10^{4}	4	1	49	2.65	8.10×10^{1}	8	6	170	-0.32	3.0
73	1.06×10^{3}	5	3	115	0.97	6.40×10^{1}	4	1	49	0.12	0.9
75	6.80×10^{2}	6	4	135	0.70	6.40×10^{1}	4	1	49	0.12	0.6
78	1.52×10^{5}	6	1	37	3.62	8.10×10^{1}	4	4	> 170	< -0.32	> 3.9
80	1.80×10^{2}	5	1	42	0.64	8.10×10^{1}	4	4	> 170	< -0.32	> 1.0
84	1.19×10^{5}	6	3	91	3.11	5.50×10^{1}	4	2	91	-0.22	3.3
88	1.36×10^{5}	5	4	200	2.83	5.50×10^{1}	4	2	91	-0.22	3.1
93	1.21×10^{5}	6	1	37	3.52	5.50×10^{1}	5	2	73	-0.12	3.6

Source: Finch et al. (1993)

Table 4-4. Ozone inactivation data of *C. parvum* oocysts suspended in phosphate buffer, 0.05 M, at 22°C

Trial No.	pH	Temp., °C	Reactor volume, mL	No. of oocysts in reactor	Applied ozone dose, mg/L	Initial ozone residual, mg/L	Final ozone residual, mg/L	Contact time, min	First order ozone decay rate, min^{-1}	Net reduction in infectivity, log-units	I.g.H. predicted reduction, log-units
16	6.9	21	50	5.0×10^5	2.6	2.7	1.4	4.9	0.13	> 2.3	3.7
17	6.9	21	50	5.0×10^5	1.8	1.8	0.9	4.9	0.14	> 2.0	2.8
30	6.9	22	50	5.0×10^5	2.1	2.0	0.9	5.0	0.17	0.7	NA
35	6.9	22	125	5.0×10^5	2.9	2.9	1.3	10.0	0.08	> 2.8	5.9
37	6.9	22	125	5.0×10^5	2.8	2.7	1.9	4.5	0.07	> 2.9	3.9
43	6.9	22	50	1.0×10^6	1.6	1.7	0.3	9.9	0.16	> 1.6	3.5
45	6.9	22	50	1.0×10^6	1.4	1.4	0.1	14.9	0.18	> 1.9	3.3
56	6.9	22	50	1.0×10^6	1.7	1.7	0.3	9.8	0.16	3.6	3.4
58	6.9	22	50	1.0×10^6	2.4	2.4	1.2	4.8	0.14	3.6	3.4
95	7.0	22	50	1.0×10^6	1.4	1.4	0.6	4.8	0.15	1.3	2.3
97	7.0	22	50	1.0×10^6	1.7	1.7	0.3	9.8	0.16	2.5	3.4
100	7.0	22	50	3.6×10^7	0.8	0.8	0.3	4.6	0.15	2.1	1.6
102	7.0	22	50	3.6×10^7	2.6	2.4	0.5	9.6	0.16	4.3	4.3
105	7.0	22	50	1.1×10^7	1.6	1.5	0.2	10.8	0.21	3.5	3.0
107	7.0	22	50	1.1×10^7	2.4	2.2	0.2	14.7	0.17	4.7	4.5
a83	6.9	23	25	5.0×10^6	1.7	1.0	0	3.4	1.70	0.8	0.7/†0.8
a210	6.9	22	25	5.0×10^6	1.1	1.2	0.4	3.8	0.31	1.0	1.7
a211	8.0	22	25	5.0×10^6	1.3	1.4	0.2	3.9	0.56	1.1	1.6
a221	8.0	22	25	5.0×10^6	1.4	1.5	0.3	3.8	0.46	1.1	1.7
a414	8.0	23	25	5.0×10^6	ND	5.7	0.1	4.7	0.95	> 3.1	3.1
a461	6.9	22	25	3.5×10^5	1.3	0.9	0.2	2.9	0.55	1.4	1.0/†1.1
a672	6.0	24	1000	3.0×10^7	0.7	0.7	0.7	3.7	0.01	2.1	1.5
a859	6.0	22	500	1.5×10^7	0.9	0.8	0.6	3.8	0.05	1.6	1.6

Source: Finch et al. (1993)

Note: NA - Not Applicable; a - additional trials conducted in this work

Table 4-5. Summary of *C. parvum* oocyst infection in neonatal CD-1 mice for ozone disinfection trials at 22°C

Trial No.	Inoculum oocysts, per animal	Live animals in cohort	Infected animals in cohort	Estimated infectious dose, oocysts per animal	Reduction in infectivity, log units	Inoculum oocysts, per control animal	Live animals in control group	Infected animals in control group	Estimated infectious dose, oocysts per animal	Reduction in control infectivity, log units	Net reduction in infectivity, log-units
16	6.00×10^{3}	8	0	< 30	> 2.30	3.00×10^{2}	9	9	> 297	< 0	> 2.3
17	2.88×10^{3}	9	1	28	2.01	3.00×10^{2}	9	9	> 297	< 0	> 2.0
35	7.29×10^{3}	6	0	< 37	> 2.30	2.10×10^{1}	5	2	73	-0.54	> 2.8
37	8.13×10^{3}	6	0	< 37	> 2.35	2.10×10^{1}	5	2	73	-0.54	> 2.9
43	6.95×10^{2}	6	0	< 37	> 1.28	1.09×10^{2}	5	5	> 200	< -0.27	> 1.6
45	2.03×10^{3}	4	0	< 49	> 1.62	1.09×10^{2}	5	5	> 200	< -0.27	> 1.9
56	1.33×10^{5}	5	1	42	3.50	6.00×10^{1}	5	2	73	-0.08	3.6
58	1.54×10^{5}	5	1	42	3.57	6.00×10^{1}	5	2	73	-0.08	3.6
95	1.08×10^{3}	5	2	73	1.17	5.50×10^{1}	5	2	73	-0.12	1.3
97	1.10×10^{4}	5	1	42	2.42	5.50×10^{1}	5	2	73	-0.12	2.5
100	1.65×10^{4}	5	4	200	1.92	7.50×10^{1}	5	3	115	-0.19	2.1
102	1.49×10^{6}	5	3	115	4.11	7.50×10^{1}	5	3	115	-0.19	4.3
105	2.02×10^{5}	5	2	73	3.45	7.50×10^{1}	4	2	91	-0.09	3.5
107	1.57×10^{6}	5	1	42	4.58	7.50×10^{1}	4	2	91	-0.09	4.7
a83	3.33×10^{2}	5	1	18	1.28	5.00×10^{1}	5	1	18	0.46	0.8
a210	5.00×10^{3}	5	3	121	1.62	5.00×10^{2}	5	3	121	0.62	1.0
a211	5.00×10^{3}	5	4	401	1.10	ND	ND	ND	ND	ND	1.1
a221	5.00×10^{2}	5	1	41	1.09	4.00×10^{1}	5	0	< 41	> -0.01	< 1.1
	5.00×10^{3}	5	4	477	1.02	4.00×10^{2}	5	5	> 477	< -0.08	> 1.1
a414	2.00×10^{5}	5	4	1334	2.18	1.50×10^{2}	5	5	> 1334	< -0.95	> 3.1
a461	1.50×10^{3}	5	2	73	1.32	1.00×10^{2}	5	3	115	-0.06	1.4

Source: Finch et al. (1993)
Note: ND - Not Determined

Table 4-6. Incomplete gamma Hom model parameter estimates and confidence limits for ozone

Data set Finch et al. (1993)	Temp., °C	No. of trials	Kinetic model	$\hat{k}$ +/- 95% limits	$\hat{m}$ +/- 95% limits	$\hat{n}$ +/- 95% limits	$\hat{\sigma}$	$\ln L_{max}$	D
Original	7	11	I.g.H.	0.48	0.72	0.79	0.43	1.61	0.15
				0.41-0.55	0.64-0.79	0.48-1.06			
Original	22	15	I.g.H.	0.82	0.65	0.42	0.79	-4.38	ND
				0.66-0.99	0.53-0.74	<0, 0.98			
Trial 30 omitted	22	14	I.g.H.	0.85	0.62	0.65	0.55	0.38	0.18
				0.72-0.99	0.52-0.70	0.16-1.09			
Trial 30 omitted	22	14	C-W	0.40	-	1.00	0.63	-0.86	ND
				ND		ND			
Arrhenius analysis	7	11	I.g.H.	0.54	0.67	0.73	0.44	1.48	ND
Arrhenius analysis	22	14	I.g.H.	0.76	0.67	0.73	0.56	0.31	ND

Note: ND - Not Determined.

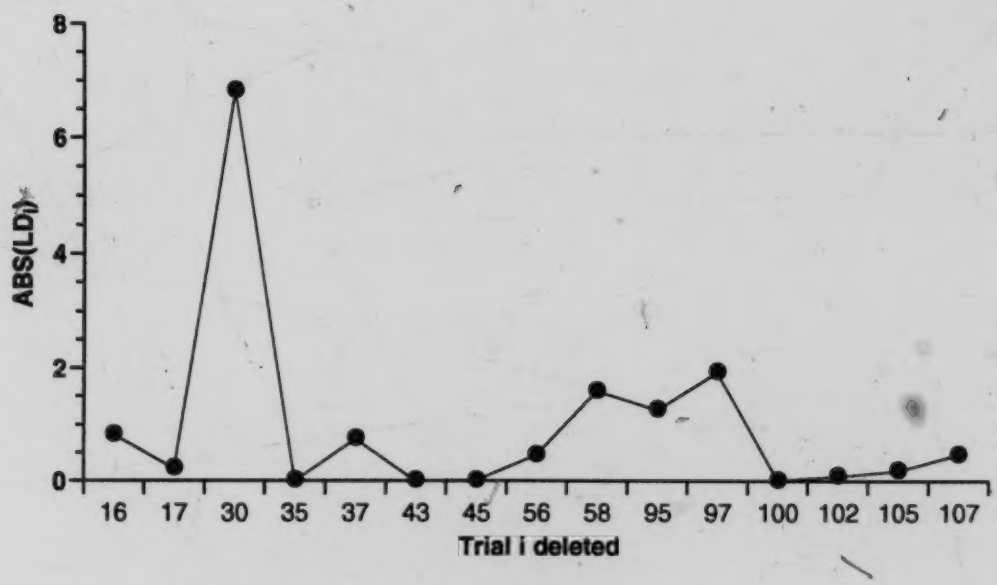

Figure 4-1. LD_i index plot for the I.g.H. model at 22°C

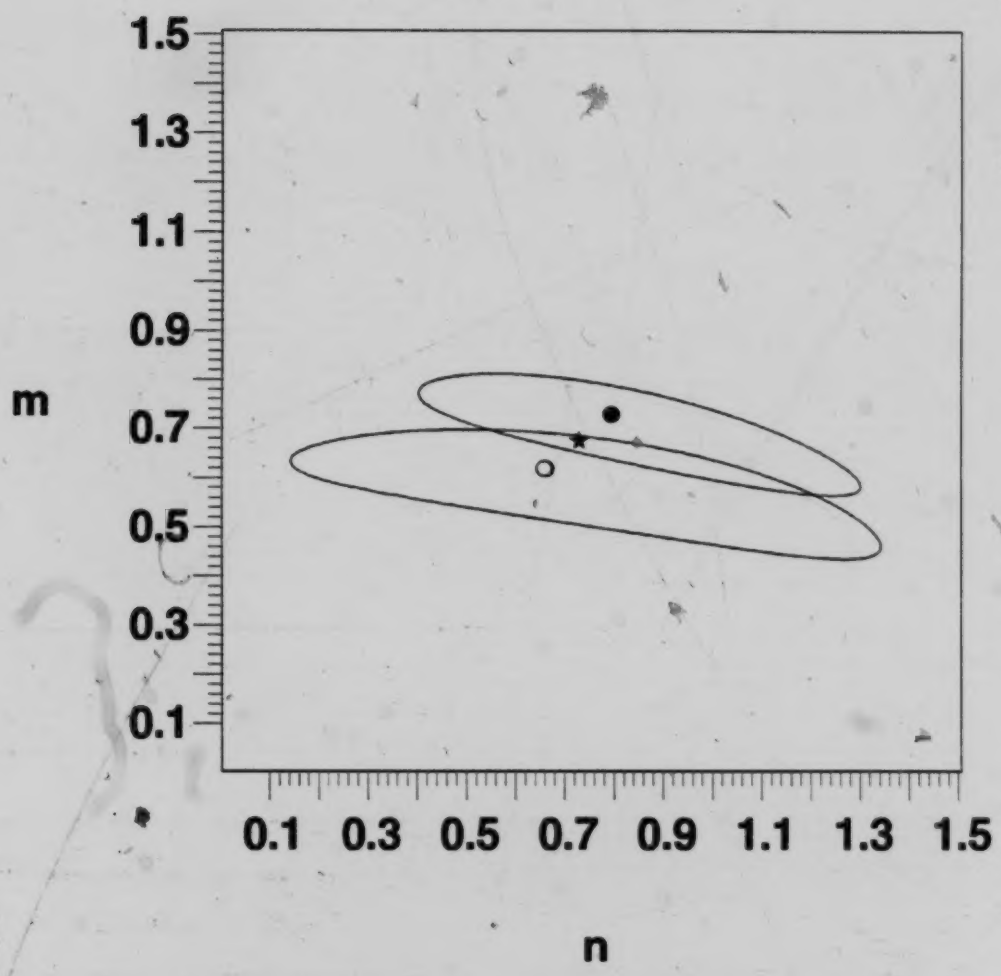

Figure 4-2. Approximate 95% profile likelihood for the m-n parameter subset (conditional for optimal $\hat{k}$) for the I.g.H. model at 7°(●) and 22°C(○). A ★ denotes the value of m and n used to calculate the Arrhenius activation energy.

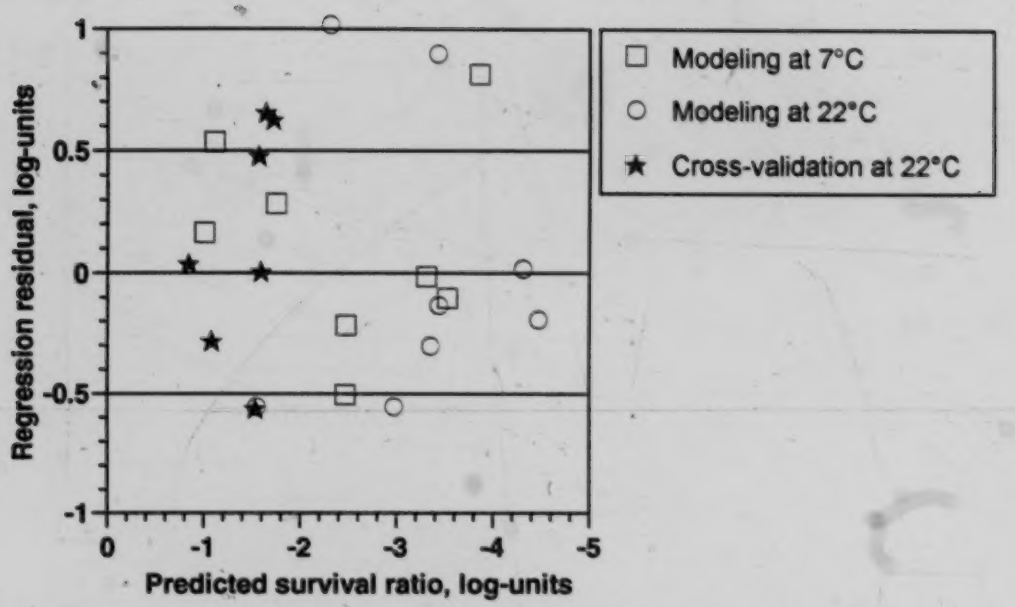

Figure 4-3. Regression residuals plotted with respect to I.g.H. model fitted survival ratios at 7° and 22°C, and cross-validation data at 22°C

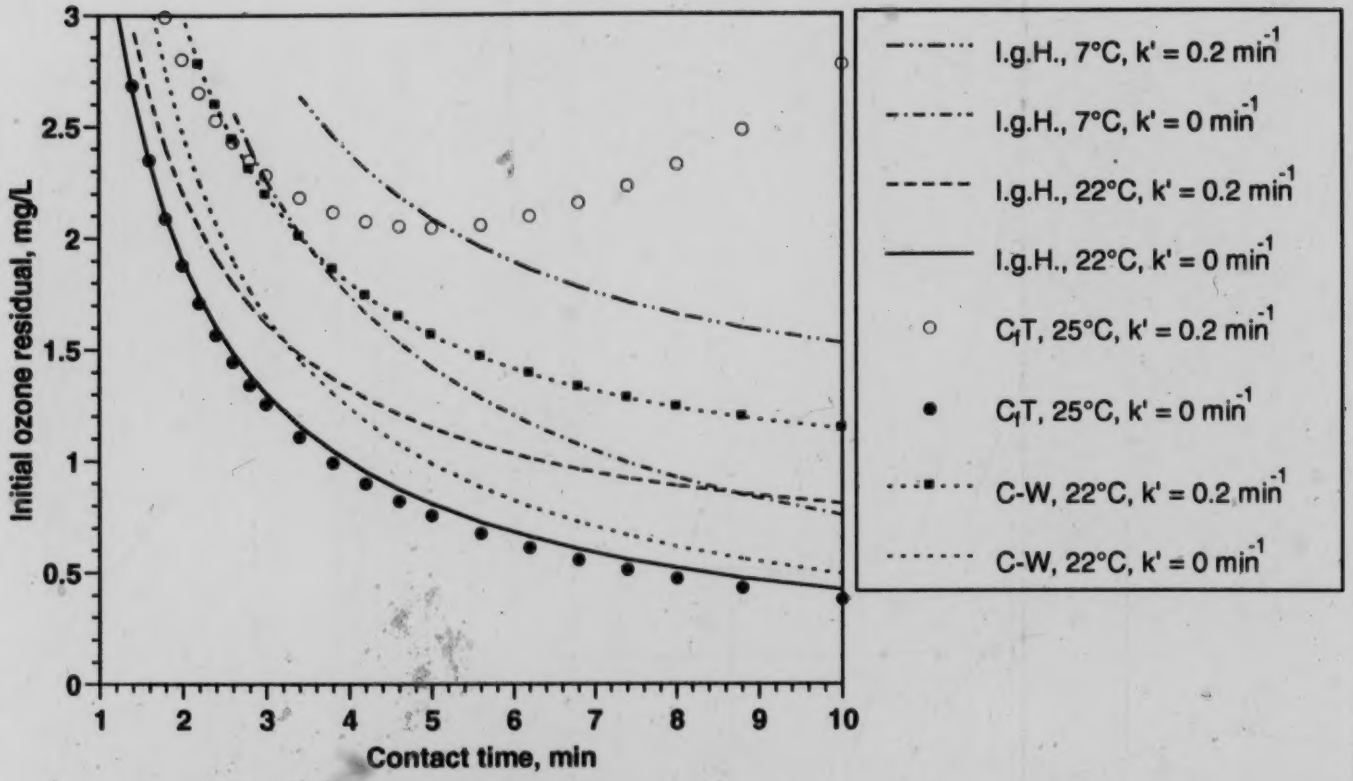

Figure 4-4. Ozone process requirements for 2.0 log-units inactivation of *C. parvum* oocysts based on the I.g.H. and C-W models for pH 6.0 to 8.0 and the CT value of 3.75 mg·min/L (pH 7, 25°C)

Chapter 5

Synergistic Effect of Sequential Exposure of *Cryptosporidium parvum* Oocysts to Ozone and Chlorine[1]

5.1 Introduction

Ozone is one of the leading disinfectants for controlling *Cryptosporidium* (Peeters et al. 1989; Korich et al. 1990; Finch et al. 1993a; Armstrong et al. 1994). Because free chlorine or monochloramine are frequently added as the final disinfectant in water treatment facilities that use ozone as the primary disinfectant, experiments were conducted on the effect of sequential exposure of *C. parvum* to ozone followed by free chlorine or preformed monochloramine (Cl_2:N weight ratio of 3:1) at pH 6.0 and 8.0, respectively, at 22-24°C. Sequential treatment by chemical compounds which provides more inactivation than expected from the additive (logarithmic) effects of single disinfectants demonstrates synergy. Sequential application of ozone followed by monochloramine has been demonstrated to provide enhanced levels of *Cryptosporidium parvum* oocyst inactivation (Finch et al. 1995). Incomplete gamma Hom (I.g.H.) models based on parameter estimates developed in Chapters 3 and 4 were used to calculate the additive effects of *C. parvum* oocyst exposure to aqueous ozone and free chlorine.

5.2 Materials and Methods

5.2.1 Oxidant Stock Solutions and Laboratory Materials

Laboratory protocols for disinfection trials conducted in this study are the same as those used in earlier *Cryptosporidium* work (Finch et al. 1993a) and described in Chapters

[1] Gyürék, L.L., G.R. Finch, and M. Belosevic paper submitted to *Journal of Env. Eng.*, (September, 1997)

3 and 4. Ozone gas was generated from extra-dry oxygen using a water-cooled, Welsbach T-816 corona discharge generator (Welsbach Ozone Systems Corporation, Sunnyvale, CA). To prepare an aqueous ozone stock solution having a concentration of approximately 20 mg/L, the ozone-oxygen gas mixture was bubbled for at least 20 min through deionized water in two 500 mL gas absorption flasks connected in series. Deionized laboratory water was obtained from either a Milli-Q® system (OM-140, Millipore Corp., Bedford, Mass.) or Elga® Ultra-pure system (FisherScientific Limited, Pittsburgh, PA). Water systems were operated at a resistivity of at least 18 MΩ/cm. Ozone demand-free water was prepared by ozonating laboratory water for at least 30 min and then boiled for at least 10 min. Ozone demand-free glassware was prepared by exposing acid-washed glassware to the aqueous ozone stock solution for at least 3 h and storage for at least 2 days at 80°C. Preparation of pH 6.0 and 8.0, 0.05 M, laboratory buffered waters involved dissolution of appropriate amounts of BDH Analar grade potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate (BDH Inc., Toronto, Ontario). Buffered laboratory waters were made ozone demand-free. Details regarding preparation of chlorine stock solutions are provided in Chapter 3. Briefly, stock solutions of free chlorine were prepared daily using sodium hypochlorite and ammonium chloride was used to prepare preformed monochloramine stock solution with a Cl_2:N weight ratio of 3:1 and approximate concentration of 150 mg/L as Cl_2. Oxidant demand-free reactors included 50 mL Nalgene® (Nalge Company, Rochester, NY) polypropylene copolymer centrifuge tubes and 1,000 mL borosilicate Wheaton Media/Lab bottles. The

limited supply of oocysts available for experiments necessitated the use of the 50 mL reactors.

5.2.2 Measurement of Oxidant(s) in Stock Solutions

The concentration of aqueous ozone in the stock solution was determined using ultraviolet spectrophotometry, a Spectronic 601 (Milton Roy) or Ultrospec 2000 UV/visible (Pharmacia Biotech Ltd.; Science Park, Cambridge, England), at 260 nm and a molar absorption coefficient of 3,300 $M^{-1}cm^{-1}$ (Hart et al. 1983). The N,N-diethylphenylenediamine (DPD) colorimetric procedure was used for assessing chlorine and monochloramine stock solutions (Greenberg et al. 1992). Total and free available chlorine Hach packets (Hach Company; Loveland, CO) were used with oxidant demand-free Fisherbrand® borosilicate glass vials. A standardization curve was prepared daily as needed using potassium permanganate, BDH AnalaR reagent grade, and oxidant demand-free glassware. Appropriate dilutions were made of high concentration samples to prevent bleaching of the DPD dye.

Forward amperometric titration using a Cl Titrimeter FisherScientific model 397 and phenylarsine oxide was also used to measure chlorine species (Greenberg et al. 1992) Section 4500 - Cl D. The rate constant for the kinetic reaction between ammonia and chlorine is 4.2×10^6 $(mol/L)^{-1}s^{-1}$ at 25°C (Morris and Isaac 1983). Based on the value of the rate constant, virtually no free chlorine should co-exist with combined chlorine. For pH 8 oxidant demand-free lab water and Cl_2:N weight ratios ≤5:1, monochloramine is the predominant species of inorganic chloramines (combined chlorine) (White 1992).

Monochloramine was the only combined species of chlorine detected in the stock solution using colorimetric DPD and amperometry.

5.2.3 Disinfection Methodology

For sequential ozone-free chlorine trials, a preparation of 3.5×10^6 and 3.0×10^7 oocysts were suspended in 25 and 1,000 mL, respectively, of pH 6.0, 0.05 M, phosphate buffer. For sequential ozone-monochloramine trials, a preparation of 5.0×10^6 *Cryptosporidium parvum* oocysts was suspended in 25 mL of pH 8.0, 0.05 M, phosphate buffer. Oocysts suspended in 50 mL Nalgene® tubes were vortexed prior to ozone addition. The concentration of the stock ozone solution was measured prior to and immediately following addition of the stock ozone solution to the reactor. A calculated volume of stock solution for the required initial disinfectant dose was added using a mass-calibrated pipette. The applied ozone dose and contact time were such that expected levels of oocyst inactivation, based on the I.g.H. model, were approximately 1.7 log-units and 0.5 to 2.4 log-units for ozone-free chlorine and ozone-monochloramine trials, respectively. Nalgene® 50 mL reactors were continuously stirred using a Benchtop Shaker Model G33 (New Brunswick Scientific, Edison, NJ) operated at 100 rpm. Teflon® (Dupont) coated magnetic stir bars were used to provide mixing in the larger reactors. Reactor vessels were covered with aluminum foil to minimize volatilization and photodecomposition of ozone. The reactor (containing oocysts) was continuously sampled in a closed loop at a flow rate of 8 mL/min. The sample was carried through a short piece of small-diameter Teflon® tubing to a 35 µl flow cell with a light path of 1 cm. A diode-array spectrophotometer (Hewlett-Packard model 8452A, Sunnyvale, CA) was

used to monitor continuously the absorbance of the solution at 260 nm. Any ozone remaining at the end of the contact time was neutralized using 1 M sodium formate (BDH Inc., AnalaR grade). The mass of sodium formate added (approx. 68 ppm) did not interfere with absorbance measurements and had no effect on *C. parvum* infectivity.

Aliquots of stock sodium hypochlorite and preformed monochloramine were applied to reactors using oxidant demand-free tips and an Oxford Macro-set and Eppendorf Digital Pipettes. The colorimetric DPD method was used to measure free chlorine and total available chlorine residuals in reactors. For 1,000 mL reactors, chlorine measurements were taken from experimental reactors which contained oocysts. For trials involving 50 mL reactors, parallel reactors not containing oocysts, to which ozone demand-free water had been added in lieu of aqueous ozone and sodium formate, were used for chlorine residual measurements. For ozone-monochloramine sequential trials, no free chlorine was detected in the 1,000 mL experimental and 50 mL parallel reactors using the DPD colorimetric procedure. At the end of the contact time, chlorine species were neutralized using 1M sodium sulfite (BDH Inc., AnalaR grade). The mass of sodium sulfite added (approx. 75 ppm) had no effect on *C. parvum* infectivity. Positive controls were used to account for any potential effect of sodium formate and sodium sulfite. All experiments were conducted at 23±1.5°C. Actual pH values for experimental trials did not deviate by more than ±0.1 pH units.

5.2.4 Parasite Methods

Parasite experimental methods are described in Chapter 3 and further detail is provided in Appendix A.

5.2.5 Data Analysis

The viability of oocysts following each disinfection trial was analyzed based on a logistic dose-response model as described elsewhere (Finch et al. 1993b). Parameter estimates for the logit dose-response model are tabulated in Chapter 3. The net survival ratio for each experimental trial was calculated by subtracting the survival ratio calculated for the positive control from that of the chemical disinfectant treated group. This accounts for any changes in viability as a result of the oocyst preparatory and experimental recovery methodologies.

The Incomplete gamma Hom (I.g.H.) model, based on parameter estimates listed in Table 5-1, was used to calculate expected levels of *C. parvum* oocyst inactivation for exposure to aqueous ozone, free chlorine, and preformed monochloramine. The I.g.H. model which can account for deviations from exponential die-off kinetics and first-order disinfectant decomposition is given by (Haas and Joffe 1994):

$$\log\frac{N}{N_o} = \frac{-mkC_o^n}{(nk')^m}\gamma(m, nk'T) \qquad m > 0,\ nk'T \geq 0 \qquad (2\text{-}19)$$

where C_o is the initial disinfectant residual at time zero, mg/L; T is the contact time, min; k' is the first-order disinfectant decay rate constant, min^{-1}; k, n and m are kinetic parameters; and $\gamma(\alpha, x)$ is the Incomplete gamma function. The Incomplete gamma function is available for various mathematical software packages including Mathcad Plus 6.0 Professional Edition (MathSoft, Inc.).

5.3 Results and Analysis

5.3.1 Ozone

Experimental data for sequential ozone-free chlorine and ozone-monochloramine trials conducted at pH 6.0 and pH 8.0, at 23±1.5°C, is summarized in Tables 5-2 and 5-4, respectively. A summary of animal infectivity data used to calculate the level of inactivation for each experimental trial is summarized in Tables 5-3 and 5-5. Note that sequential ozone-free chlorine trials 645 to 647 and 673 to 679 are serially correlated, i.e. free chlorine residuals were measured at various contact times and 200 mL samples extracted from the 1,000 mL reactor to assess the reduction in oocyst infectivity. The use of the 50 mL Nalgene® reactors resulted in relatively high oocyst recoveries but also higher instantaneous ozone demands, i.e. the loss of ozone which occurs within seconds of stock ozone dosing, and a relatively faster rate of ozone disappearance during the applied contact time compared to the 1,000 mL reactors, particularly at pH 6.0. The level of *C. parvum* oocyst inactivation attributable to an instantaneous ozone demand ≤0.7 mg/L was shown in Chapter 4 to be negligible, i.e. <0.1 log-units. The applicability of the I.g.H. model to describe *C. parvum* inactivation kinetics for first-order ozone decay rates ranging from 0.01 to 1.7 min^{-1} in laboratory water at pH 6.0 to 8.0 was demonstrated in Chapter 4 using cross-validation trials. For the sequential ozone-monochloramine trials at pH 8.0, the use of 50 mL Nalgene® reactors resulted in first-order ozone decay rates ranging from 0.3 to 1.7 min^{-1}. In comparison, the rate of ozone decomposition in 1,000 mL reactors under similar conditions was measured to be 0.27 min^{-1}. The expected level of *C. parvum* oocyst inactivation attributable to ozone pretreatment was estimated vis-à-vis the I.g.H.

model based on the measured initial ozone residual, contact time and first-order ozone decay rate constant for each experimental trial. The cross-validation trials presented in Chapter 4 suggest the actual level of *C. parvum* oocyst inactivation will frequently be within ±0.6 log-units of the I.g.H. model predicted survival ratio for ozone exposure in batch reactors and laboratory water.

5.3.2 Free Available Chlorine

Experimental data describing the sequential application of free chlorine to reactors following ozone pretreatment at pH 6.0 is summarized in Table 5-2. Initial chlorine demand varied from 0.22 to 0.54 mg/L based on residual measurements in 1,000 mL reactors in which oocysts were ozonated. In comparison, the initial chlorine demand measured in 1,000 mL reactors containing oocysts not exposed to ozone pretreatment was found to be ≤0.1 mg/L (Chapter 3). For the 50 mL Nalgene® parallel reactors not containing oocysts, and to which ozone was not applied, the initial chlorine demand varied from 0.12 to 0.56 mg/L. Chlorine demand consists of chemical reaction and volatilization demands as well as losses associated with oocysts. The expected level of *C. parvum* oocyst inactivation attributable to free chlorine exposure at pH 6.0, at which hypochlorous acid constitutes approximately 97.5% of the free chlorine (White 1992), was estimated vis-à-vis the I.g.H. model based on the applied free chlorine dose, contact time and the assumption of no chlorine decomposition. A summary of the level of *C. parvum* oocyst inactivation expected for a given free chlorine exposure is summarized in Table 5-2. Note that accounting for initial chlorine demand and a typical first-order decay rate constant of 3×10^{-4} min^{-1} decreases I.g.H. model predicted values by ≤0.1 log-units.

5.3.3 Preformed Monochloramine

Experimental data describing the sequential application of preformed monochloramine to reactors following ozone pretreatment at pH 8.0 is summarized in Table 5-4. The initial monochloramine demand for ozone pretreated oocysts suspended in a 1,000 mL reactor was measured to be ≤0.1 mg/L. A similar result was observed in Chapter 3 for 500 mL reactors containing oocysts not ozone pretreated. For the parallel 50 mL Nalgene® reactors (not containing oocysts) in this study, the initial monochloramine demand was variable but most often negligible. The precision associated with DPD measurements in addition to dilution factors was responsible for some initial monochloramine residuals listed in Table 5-4 to be apparently greater than the applied monochloramine dose. This discrepancy was problematic to 50 mL reactors because of the necessarily smaller sampling volumes used for residual measurements. As shown in Table 5-4, a number of trials had initial monochloramine demands measured in parallel reactors which were greater than expected. These trials were nonetheless included in the assessment of synergistic effects because these monochloramine losses are not significant with respect to the (expected) level of *C. parvum* oocyst inactivation. Inactivation attributable to preformed monochloramine (3:1) at pH 8.0 was estimated vis-à-vis the I.g.H. model based on the applied monochloramine dose, contact time and the assumption of no monochloramine losses. Based on this conservative assumption, the expected level of *C. parvum* oocyst inactivation attributable to monochloramine exposure was <0.01 log-units for all of the experimental trials. For this reason I.g.H. model predictions are not provided in Table 5.4.

5.3.4 Ozone-Free Chlorine Synergism

Sequential treatment by chemical compounds which provides more inactivation than expected from the additive (logarithmic) effects of single compounds demonstrates synergy. Table 5-2 summarizes the synergistic effect of sequential exposure of *C. parvum* oocysts to ozone followed by free chlorine at pH 6.0. The magnitude of the synergistic effect is an indication of the extent to which the efficacy of the free chlorine, predominantly hypochlorous acid, to inactivate *C. parvum* oocysts has been enhanced, or "synergized", by ozone pretreatment. Synergistic effects tabulated in Table 5-2 are plotted with respect to the level of ozone pretreatment (i.e. expected level of inactivation caused by ozone pretreatment), free chlorine CT value based on the applied dose, and free chlorine contact time, in Figures 5-1 to 5-3, respectively. In Figure 5-1, experimental trials are grouped based on the CT value of free chlorine exposure. Up and down arrows illustrated in Figure 5-1 denote greater than and less than synergistic effects, respectively. Given the uncertainty associated with the I.g.H. model used to calculate the expected level of inactivation provided by ozone pretreatment, synergistic effects <0.6 log-units in magnitude are considered indistinguishable from zero. Figure 5-1 suggests that increasing the level of ozone pretreatment from 1.5 to 1.9 log-units does not increase the synergistic effect of sequential exposure to free chlorine for a given CT value. As well, a significant synergism, i.e. >0.6 log-units, was only found consistently for free chlorine CT values >2,100 mg·min/L independent of the ozone pretreatment level (1.5 to 1.9 log-units), as illustrated in Figure 5-2. For the free chlorine applied dose range of 0.5 to 2.5 mg/L examined in this study, it is evident from Figure 5-3 that an free chlorine contact time

>300 min, and perhaps as much as 900 min, is necessary in order for the efficacy of free chlorine to inactivate *C. parvum* oocysts to be significantly enhanced by prior ozone treatment at pH 6.0 and 24°C. This data suggests that for free chlorine residuals typical of water distribution systems (<2.5 mg/L) and contact times approaching 1,000 min, nearly 4 log-units of *C. parvum* inactivation is possible given an ozone pretreatment which alone provides 1.5 log-units inactivation.

5.3.5 Ozone-Preformed Monochloramine Synergism

Table 5-4 summarizes the synergistic effect of sequential exposure of *C. parvum* oocysts to ozone followed by preformed monochoramine at pH 8.0. Synergistic effects tabulated in Table 5-4 are plotted with respect to the level of ozone pretreatment (i.e. expected level of inactivation caused by ozone pretreatment), monochloramine CT value based on the applied dose, and monochloramine contact time, in Figures 5-4 to 5-6, respectively. In Figure 5-4, experimental results are grouped according to monochloramine CT values. Synergistic effects plotted within the dashed line envelope corresponding to ±0.6 log-units synergism are considered indistinguishable from zero because of I.g.H. model uncertainty. For sequential (preformed) monochloramine CT values >100 mg·min/L, the efficacy of monochloramine to inactivate *C. parvum* oocysts was enhanced by 0.6 to 1.9 log-units, independent of the ozone pretreatment level (0.6 to 2.4 log-units) as illustrated in Figure 5-4. No consistent synergistic effect was found for monochloramine CT values <100 mg·min/L as shown in Figure 5-5. For the applied monochloramine doses ranging from 0.7 to 2.1 mg/L examined in this study, it is evident from Figure 5-6 that a monochloramine contact time ≥100 min is required in order for the

efficacy of monochloramine to inactivate *C. parvum* oocysts be significantly enhanced by prior ozone treatment at pH 8.0 and 23°C. This data suggests that for monochloramine residuals and contact times typical of water distribution systems, <2.5 mg/L and ≥100 min, respectively, >3 log-units of *C. parvum* inactivation is possible given an ozone pretreatment providing at least 1.5 log-units inactivation.

5.3.6 Synergism

For a given level of ozone pretreatment, the level of *C. parvum* inactivation provided by sequential exposure to <2.5 mg/L of monochloramine or free chlorine is similar at pH 8.0 and pH 6.0, respectively. The disinfection efficiency of sequentially applied monochloramine is however greater than that of free chlorine because the required free chlorine contact time is >300 min compared to ≥100 min for monochloramine. Free chlorine at pH 6.0 was demonstrated in Chapter 3 to be significantly more effective than monochloramine at pH 8.0 to inactivate *C. parvum* (without oxidant pretreatment) at low concentrations, i.e. <5 mg/L. Exposure of *C. parvum* oocysts to aqueous ozone therefore enhances the efficacy of monochloramine to a greater extent than for free chlorine.

There have been reports in the literature of synergism between disinfectant species leading to more effective treatment. Most studies have reported the results from a mixture of chemical disinfectants used simultaneously (Kouame and Haas 1991; Venczel et al. 1996). Considering sequential application, it was shown in Chapter 3 that free chlorine followed by monochloramine (post-ammoniation) at pH 8.0 provided synergistic enhancement of the disinfection efficiency of monochloramine (3:1) for *C. parvum* oocysts. Increasing levels of free chlorine pretreatment reduced the subsequent

monochloramine concentration and contact time necessary for a given level of inactivation. It was postulated in Chapter 3 that the permeability of the oocyst wall to monochloramine increases proportionally with respect to the level of free chlorine pretreatment, which itself provided no measurable reduction in infectivity, permitting monochloramine to more easily diffuse through the oocyst wall and damage sporozoites. The synergistic effect of sequential exposure to free chlorine or monochloramine was found in this study to not be proportional to the level of ozone pretreatment for the levels investigated (0.5 to 2.4 log-units inactivation).

A previous study investigated the inactivation of *C. parvum* oocysts using ozone followed by free chlorine (Ransome et al. 1993). Approximately 1×10^6 oocysts were suspended in 1L of pH 7.0 buffered spring water at 10°C. Ransome and co-workers recovered oocysts following disinfection using a polycarbonate filter and centrifugation at 1,500×*g* for 10 min. For an initial ozone residual of 1.2 mg/L applied for 10 min followed by an initial chlorine concentration of 0.5 mg/L applied for 30 min, *in vitro* excystation estimated an 85% reduction (control adjusted) in oocyst viability. No animal infectivity data is available for this reported trial which is an average of four experiments. For this level of pretreatment, ozone alone was reported to give an 80 to 90% reduction in excystation. It was concluded that the use of ozone and chlorine was no more effective than using ozone alone (for the concentrations and contact times examined). The experimental data reported by Ransome and co-workers (1993) is substantiated by the present study which demonstrated that a free chlorine contact time >300 min, and perhaps

as high as 900 min, is necessary in order for free chlorine to have enhanced disinfection efficiency.

5.4 Conclusions

Exposure of *C. parvum* oocysts to aqueous ozone was demonstrated to enhance the disinfection efficiency of sequentially applied free chlorine at pH 6.0 or preformed monochloramine (3:1) at pH 8.0 and 23±1°C, provided appropriate contact time are used. Following ozone pretreatment, the required contact time of free chlorine and preformed monochloramine was >300 min (perhaps as high as 900 min) and ≥100 min, respectively, in order to demonstrate synergy. Free chlorine residuals typical of water distribution systems, i.e. <2.5 mg/L, and contact times approaching 1,000 min may provide >3.5 log-units of *C. parvum* oocyst inactivation given an ozone pretreatment level of 1.5 log-units inactivation. Sequential application of preformed monochloramine may provide >3.0 log-units inactivation for contact times ≥100 min. The use of ozone pretreatment followed by chlorine species, in particular monochloramine, gives water utilities an effective disinfection method to waterborne control *Cryptosporidium*. Additional work is needed to validate the observed synergistic effects for water quality conditions found in practice.

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Table 5-1. Incomplete gamma Hom model parameter estimates

Disinfectant	pH	$\hat{k}$	$\hat{m}$	$\hat{n}$	Model Constraints: C = Conc. (mg/L) T = Time (min)
Ozone	6.0-8.0	0.85	0.62	0.65	$0.7 \le C \le 2.9$ $3 \le T \le 15$
Free Available Chlorine	6.0	1.1×10^{-2}	0.50	0.80	$1.7 \le C \le 17$ $60 \le T \le 1030$
Preformed Monochloramine (3:1)	8.0	1.1×10^{-6}	1.28	2.53	$2 \le C \le 15$ $60 \le T \le 1000$

Table 5-2. Sequential ozone-free chlorine inactivation data of *C. parvum* oocysts suspended in phosphate buffer, 0.05 M, pH 6.0, and 24°C

Trial No.	Reactor vol., mL	No. of oocysts in reactor	Applied ozone dose, mg/L	Initial ozone residual, mg/L	Final ozone residual, mg/L	Ozone contact time, min	First-order ozone decay rate constant, min^{-1}	I.g.H. predicted inactivation by ozone, log-units	Applied Cl_2 dose, mg/L	Initial Cl_2 residual mg/L	Final Cl_2 residual mg/L	Cl_2 contact time, min	I.g.H. predicted inactivation by Cl_2, log-units	Observed net reduction i infectivity, log-units	Synergistic effect, log-units
454	25	3.5×10^6	1.36	1.25	0.20	4.03	0.401	1.64	0.50	0.38	0.03	240	0.10	1.37	-0.37
456	25	3.5×10^6	1.24	1.04	0.34	4.23	0.264	1.65	1.00	0.48	0.40	240	0.17	1.71	-0.11
458	25	3.5×10^6	1.22	1.14	0.40	4.00	0.262	1.72	1.99	1.43	1.33	240	0.29	>2.13	>0.12
645	1000	3.0×10^7	0.96	0.99	0.93	3.58	0.017	1.83	2.50	2.21	2.04	60	0.18	1.54	-0.47
646	1000	3.0×10^7	0.96	0.99	0.93	3.58	0.017	1.83	2.50	2.21	1.95	265	0.37	<2.02	<-0.18
647	1000	3.0×10^7	0.96	0.99	0.93	3.58	0.017	1.83	2.50	2.21	1.82	940	0.70	>3.42	>0.89
675	1000	3.0×10^7	0.75	0.74	0.71	3.65	0.011	1.54	2.50	1.96	1.90	60	0.18	>1.98	>0.26
677	1000	3.0×10^7	0.75	0.74	0.71	3.65	0.011	1.54	2.50	1.96	1.80	265	0.37	>2.90	>0.99
679	1000	3.0×10^7	0.75	0.74	0.71	3.65	0.011	1.54	2.50	1.96	1.67	860	0.67	3.90	1.69

Table 5-3. Summary of *C. parvum* oocyst infection in neonatal CD-1 mice for sequential ozone-free chlorine trials

Trial No.	Inoculum oocysts, pe animal	Live animals in cohort	Infected animals in cohort	Estimated infectious dose, oocysts per animal	Reduction in infectivity, log units	Inoculum oocysts, per control animal	Live animals in control group	Infected animals in control group	Estimated infectious dose, oocysts per animal	Reduction in control infectivity, log units	Net reductio in infectivity log-units
454	2.00×10^4	5	3	514	1.59	150	5	1	90	0.22	1.37
456	2.00×10^4	5	2	234	1.93	150	5	1	90	0.22	1.71
458	2.00×10^4	5	0	<90	>2.35	150	5	1	90	0.22	>2.13
645	5.00×10^3	5	4	165	1.48	70	10	6	81	-0.06	1.54
646	1.50×10^4	5	5	>165	<1.96	70	10	6	81	-0.06	<2.02
647	5.00×10^4	5	0	<22	>3.36	70	10	6	81	-0.06	>3.42
675	5.00×10^3	1	0	<60	>1.92	70	10	6	81	-0.06	>1.98
677	1.50×10^4	5	0	<22	>2.84	70	10	6	81	-0.06	>2.90
679	1.50×10^5	5	1	22	3.84	70	10	6	81	-0.06	3.90

Table 5-4. Sequential ozone-monochloramine inactivation data of 5×10^6 *C. parvum* oocysts suspended in 25 mL, 0.05M, phosphate buffer at pH 8.0

Trial No.	Temp., °C	Applied ozone dose, mg/L	Initial ozone residual, mg/L	Final ozone residual, mg/L	Ozone contact time, min	First-order ozone decay rate constant, min^{-1}	I.g.H. predicted inactivation by ozone, log-units	Applied NH_2Cl dose, mg/L	Initial NH_2Cl residual, mg/L	Final NH_2Cl residual, mg/L	NH_2Cl contact time, min	Observed net reduction in infectivity, log-units	Synergistic effect, log-units
87	22	2.00	1.10	0	3.63	1.72	0.75	2.00	1.91	1.74	240	>2.28	>1.52
106	23.5	1.38	1.24	0	5.23	0.67	1.40	2.00	1.96	1.88	240	1.98	0.57
108	23.5	1.37	1.05	0	5.03	0.68	1.24	2.00	1.96	1.88	240	1.89	0.64
141	23	2.66	1.98	0.22	4.63	0.40	2.30	2.00	1.53	1.42	120	3.37	1.07
142	23	1.69	1.64	0.15	4.37	0.54	1.79	1.00	1.18	1.22	95	3.46	1.67
145	23.5	0.55	0.38	0.21	1.97	0.30	0.60	1.50	0.84	0.84	30	1.34	0.74
147	23.5	0.63	0.65	0.26	2.03	0.40	0.82	1.50	0.64	0.64	10	1.27	0.45
150	23.5	0.56	0.56	0.20	2.08	0.53	0.72	0.75	0.37	0.35	30	0.03	-0.69
289	23	0.73	0.61	0.03	2.48	1.38	0.56	1.15	0.84	0.70	240	1.10	0.54
291	23	0.72	0.62	0.02	2.53	1.47	0.55	2.10	1.91	1.67	240	2.13	1.58
292	23	2.07	1.80	0	5.00	1.00	1.44	1.15	0.57	0.60	11.5	<2.10	<0.66
295	23	2.01	1.76	0	5.00	0.89	1.51	2.10	2.02	1.91	240	3.37	1.86
309	23	2.08	1.74	0	5.00	0.89	1.50	1.00	0.83	0.83	240	<2.93	<1.43
312	23	2.04	1.46	0	5.00	0.91	1.32	2.10	2.04	2.04	10	<1.71	<0.39
314	23	2.19	1.75	0.04	4.82	0.77	1.61	1.00	0.90	0.90	10	2.70	1.09
317	23	0.71	0.47	0.04	2.40	1.14	0.52	2.10	2.26	2.40	10	<0.81	<0.29
331	23	2.10	1.52	0	5.00	1.25	1.13	0.65	0.49	0.49	240	1.83	0.70
333	23	2.20	1.74	0	5.00	1.44	1.14	0.65	0.49	0.49	10	1.57	0.43
335	23	2.03	1.80	0	5.00	1.31	1.23	2.00	1.82	1.86	10	1.19	-0.04
347	22.5	2.72	2.56	0.12	4.93	0.55	2.44	1.00	1.02	1.04	60	2.57	0.13
350	22.5	3.12	2.68	0.13	4.85	0.77	2.14	2.00	1.97	2.06	30	2.88	0.74

Table 5-5. Summary of *C. parvum* oocyst infection in neonatal CD-1 mice for sequential ozone-monochloramine trials

Trial No.	Inoculum oocysts, pe animal	Live animals in cohort	Infected animals in cohort	Estimated infectious dose, oocysts per animal	Reduction in infectivity, log units	Inoculum oocysts, per control animal	Live animals in control group	Infected animals in control group	Estimated infectious dose, oocysts per animal	Reduction in control infectivity, log units	Net reduction i infectivity, log-units
87	1.88×10^4	5	0	<17	>3.03	249	5	2	44	0.75	>2.28
106	3.38×10^4	5	4	401	1.93	40	5	2	45	-0.05	1.98
108	2.78×10^4	5	4	401	1.84	40	5	2	45	-0.05	1.89
141	3.21×10^4	5	1	14	3.37	400	5	4	401	0	3.37
142	3.91×10^4	5	1	13	3.46	400	5	4	401	0	3.46
145	4.56×10^3	5	5	>401	<1.06	369	5	4	401	-0.04	1.34
	4.56×10^2	5	0	<14	>1.53	369	5	4	401	-0.04	
147	4.03×10^3	5	5	>401	<1.00	369	5	4	401	-0.04	1.27
	4.03×10^2	5	0	<14	>1.47	369	5	4	401	-0.04	
150	3.97×10^2	5	4	401	-0.01	369	5	4	401	-0.04	0.03
289	2.50×10^3	5	4	477	0.72	200	5	4	477	-0.38	1.10
291	2.50×10^3	5	0	<41	>1.79	200	5	4	477	-0.38	2.13
	2.50×10^4	5	5	>477	<1.72	200	5	4	477	-0.38	
292	2.50×10^4	5	5	>477	<1.72	200	5	4	477	-0.38	<2.10
295	4.60×10^5	5	4	477	2.99	200	5	4	477	-0.38	3.37
309	2.00×10^5	5	5	>477	<2.62	180	4	3	369	-0.31	<2.93
312	1.20×10^4	5	5	>477	<1.40	180	4	3	369	-0.31	<1.71
314	1.00×10^4	5	1	41	2.39	180	4	3	369	-0.31	2.70
317	1.50×10^3	5	5	>477	<0.50	180	4	3	369	-0.31	<0.81
331	2.50×10^4	4	3	369	1.83	200	5	3	199	0	1.83
333	1.50×10^3	5	1	41	1.57	200	5	3	199	0	1.57
335	1.50×10^3	5	2	97	1.19	200	5	3	199	0	1.19
347	1.50×10^4	5	1	41	2.57	200	5	3	199	0	2.57
350	1.50×10^5	5	3	199	2.88	200	5	3	199	0	2.88

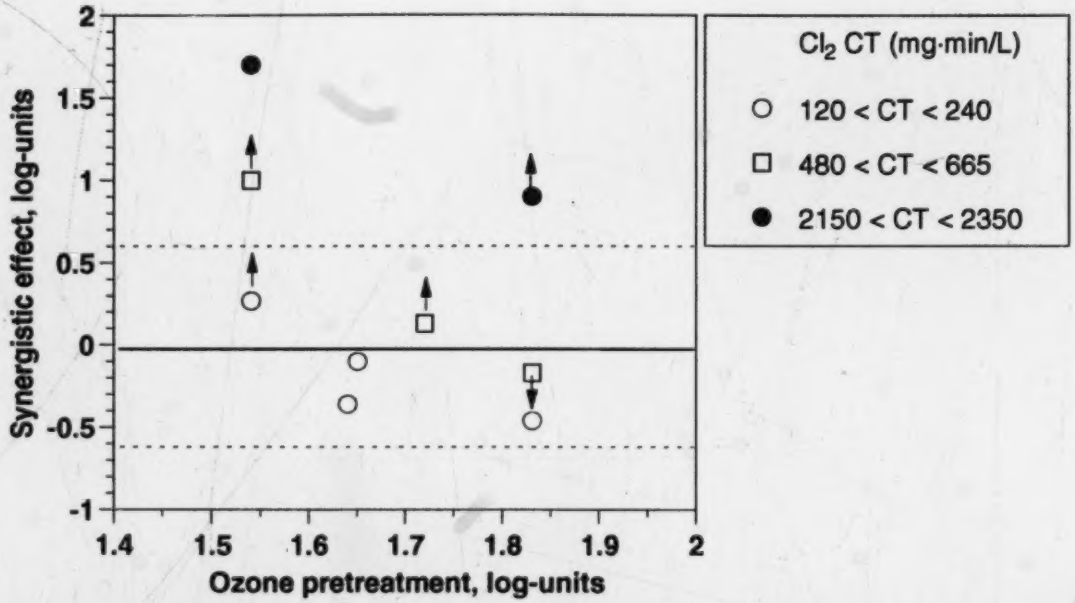

Figure 5-1. Synergistic effect of sequential exposure of *C. parvum* oocysts to ozone and free chlorine, at pH 6.0 and 24°C, illustrated as a function of the ozone pretreatment level, i.e. expected level of inactivation based on the I.g.H. model, for a specified free chlorine CT level.

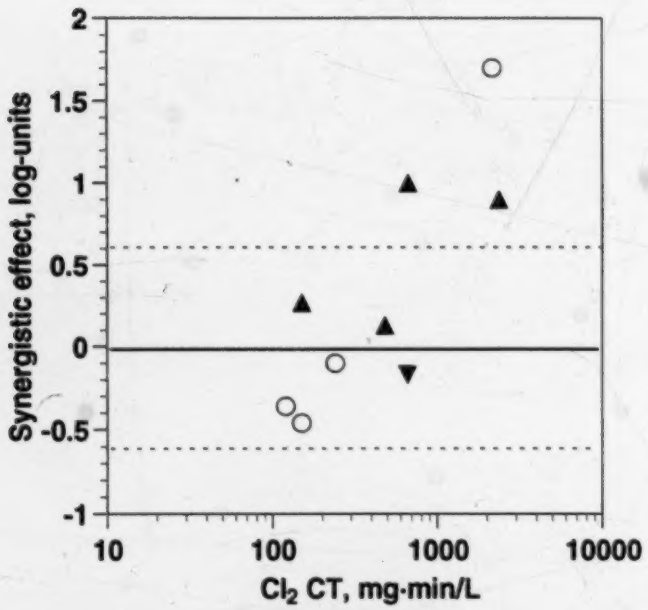

Figure 5-2. Synergistic effect of sequential exposure of *C. parvum* oocysts to ozone and free chlorine, at pH 6.0 and 24°C, illustrated as a function of the free chlorine CT level. Less than or greater than synergistic effects are represented by ▼ and ▲, respectively.

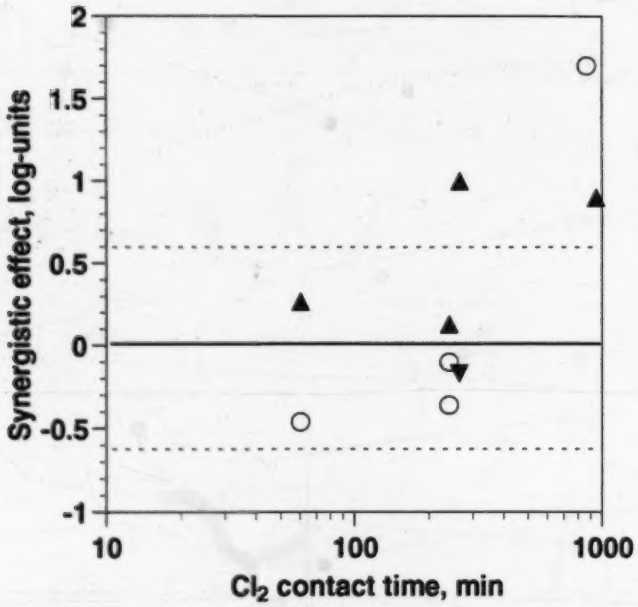

Figure 5-3. Synergistic effect of sequential exposure of *C. parvum* oocysts to ozone and free chlorine, at pH 6.0 and 24°C, illustrated as a function of the free chlorine contact time. Less than or greater than synergistic effects are represented by ▼ and ▲, respectively.

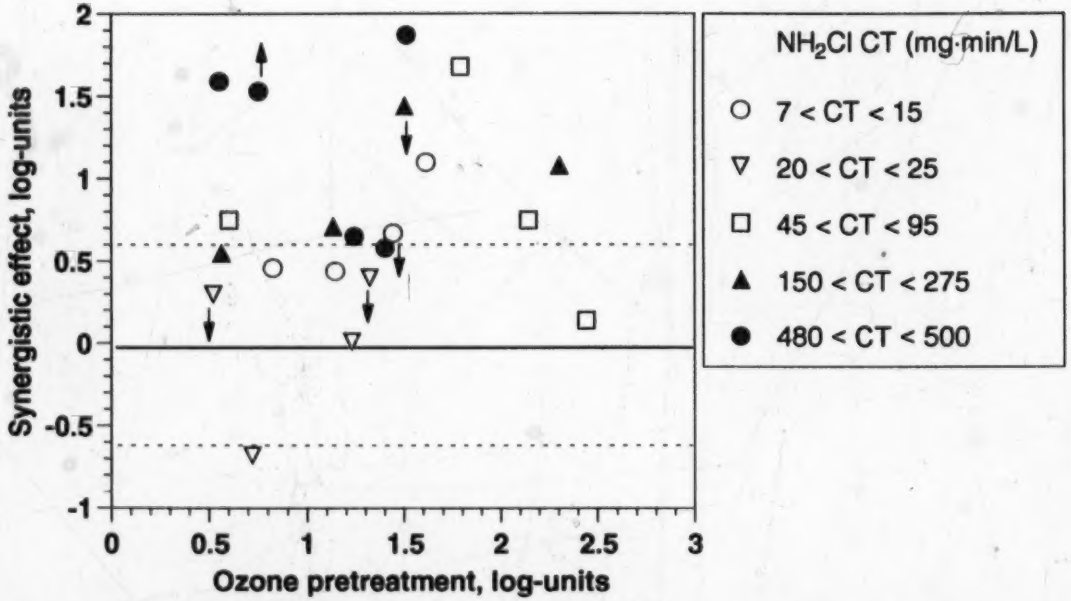

Figure 5-4. Synergistic effect of sequential exposure of *C. parvum* oocysts to ozone and preformed monochloramine (3:1), at pH 8.0 and 23°C, illustrated as a function of the ozone pretreatment level, i.e. expected level of inactivation based on the I.g.H. model, for a specified monochloramine CT level.

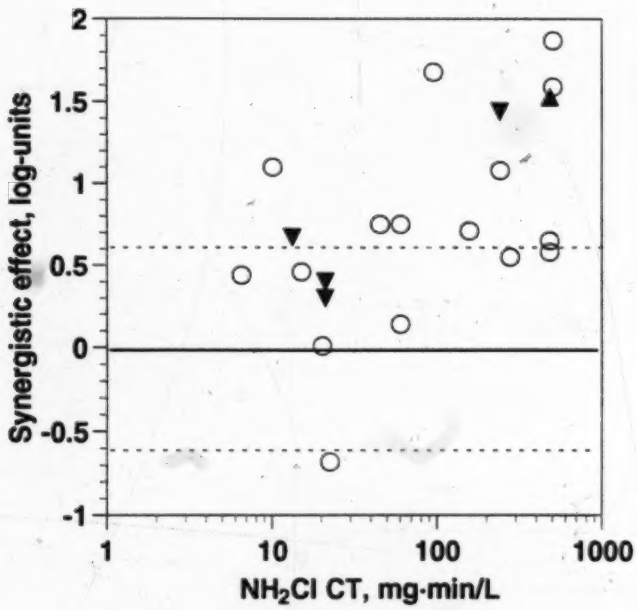

Figure 5-5. Synergistic effect of sequential exposure of *C. parvum* oocysts to ozone and preformed monochloramine (3:1), at pH 8.0 and 23°C, illustrated as a function of the monochloramine CT level. Less than or greater than synergistic effects are represented by ▼ and ▲, respectively.

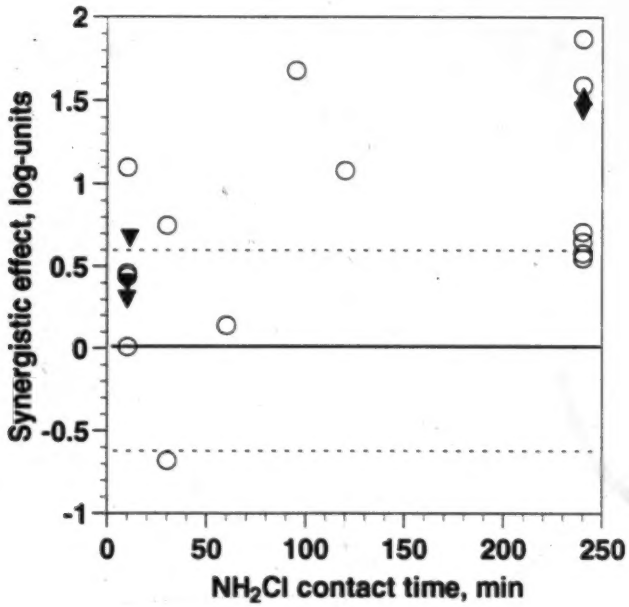

Figure 5-6. Synergistic effect of sequential exposure of *C. parvum* oocysts to ozone and preformed monochloramine (3:1), at pH 8.0 and 23°C, illustrated as a function of the monochloramine contact time. Less than or greater than synergistic effects are represented by ▼ and ▲, respectively.

Chapter 6

General Summary and Conclusions

6.1 General Overview

Cryptosporidium parvum is a coccidian protozoan that is recognized as a cause of waterborne disease in humans (Barer and Wright 1990). *C. parvum* oocysts can survive prolonged periods of time in the environment and they are considered to be present in 65 to 97% of surface waters in the United States (D'Antonio et al. 1985; Gallaher et al. 1989). *C. parvum* outbreaks from surface water supplies have been documented worldwide including Canada, the United States and Great Britain (Hayes et al. 1989; Pett et al. 1994; Rush et al. 1990). A multiple barrier approach using watershed protection, pretreatment, filtration, and disinfection is necessary to prevent transmission of *C. parvum* oocysts from surface waters supplies. Given the size of *C. parvum* oocysts, 2 to 5 μm in diameter (Soave and Armstrong 1986), and site specific conditions (quality of raw water, plant design, operational procedures), *C. parvum* oocysts may breach treatment filters making (post-) disinfection the last barrier to waterborne transmission of the human pathogen.

Cryptosporidium is not currently regulated in the U.S. but inactivation-removal requirements for *Cryptosporidium* are likely to be included in the Enhanced Surface Water Treatment Rule (ESWTR). Occurrence data for *Cryptosporidium* in source waters collected under the Information Collection Rule (ICR) will be used to determine the level of removal-inactivation required. The resistance of oocysts to inactivation by chemical agents requires the application of effective disinfectants in conjunction with appropriate

design criteria to ensure an acceptable level of risk of infection and to minimize the formation of disinfection by-products.

The goal of this research was to evaluate the effect of chemical disinfectants used in practice by the water treatment industry to control *C. parvum* in drinking water. The disinfection efficiency of free chlorine at pH 6 and 8, preformed monochloramine (chlorine to nitrogen weight ratio of 3:1) at pH 8, chloramination using post-ammoniation at pH 8, ozone at pH 6 to 8, and ozone followed by either free chlorine at pH 6 or preformed monochloramine at pH 8 was evaluated at 22°C. Bench-scale experiments were conducted using batch reactors, laboratory water, and animal infectivity to measure changes in oocyst viability. The results of this work can help to assess *C. parvum* inactivation credit for water treatment plant disinfection systems.

A kinetic inactivation model that can describe the various types of microbial survival curves, in conjunction with an appropriate data set permitting accurate estimation of model parameters, is essential to be able to evaluate the effect of a disinfectant on a microorganism. The adequacy of various kinetic inactivation models to describe ozone inactivation of heterotrophic plate count (HPC) bacteria was evaluated. Inactivation behavior of HPC bacteria by ozone was characterized by a "tailing-off" effect which has similarly been observed for *Giardia* and *Cryptosporidium* (Finch et al. 1993; Labatiuk et al. 1992). The robust three parameter I.g.H. model was found to best fit the disinfection data. The Chick-Watson model did not adequately describe the inactivation kinetics.

The SWTR requires 3 log-units removal-inactivation of *Giardia* and awards disinfection credit on the basis of CT products (characteristic disinfectant concentration ×

characteristic contact time) derived from Chick-Watson kinetics assuming a constant disinfectant concentration. Disinfectant demand-free conditions are unlikely for most oxidants and natural waters (Hoff 1987). To remedy this problem, the SWTR assumes that the characteristic disinfectant concentration is the effluent concentration from the reactor (Lev and Regli 1992a). This approach by the SWTR can lead to overdesign of disinfection systems.

A rational approach for regulatory design criteria is to use the I.g.H. model for a given level of inactivation and set of environmental conditions (pH and temperature). This model offers accurate initial disinfectant residual and contact time settings which can be graphically presented as a process design chart. In addition to the inactivation rate law and a decreasing disinfectant residual, a third issue regarding the design or assessment of disinfection contactors involves reactor hydraulics. The SWTR indirectly accounts for non-ideal reactor flow conditions by using T_{10} as the characteristic contact time (Lev and Regli 1992b). Hom-type models can be coupled with hydraulic dispersion models, such as the N-CSTR or axial dispersion model, to account for non-ideal hydraulics.

Free (available) chlorine at pH 6 was found to have an affect on *C. parvum* oocyst and can provide up to a 0.5 log-unit inactivation under practical water treatment plant conditions at 22°C. At pH 8 virtually no inactivation was observed when oocysts were exposed to free chlorine concentrations ranging from 4.9 to 80 mg/L and contact times of 48 to 245 min. Hypochlorous acid constitutes 97.5, 79.3, and 27.7% of the free chlorine at pH 6, 7, and 8, respectively (White 1992). Hypochlorous acid is thus a significantly better chlorine species for inactivation of *C. parvum* than is hypochlorite ion.

Hypochlorite ion is a poor disinfectant presumably because of it's negative electrical charge which inhibits it's diffusion across biological membranes. Application of monochloramine alone at pH 8 and 22°C is not effective against *C. parvum* because of an apparent threshold concentration requiring concentrations to be >10 mg/L.

I.g.H. models were developed for chloramination trials grouped according to applied chlorine pretreatment CT products of 4 to 11, 20 to 85, and 725 to 2,200 mg·min/L. Post-ammoniation at pH 8 was found to be more effective in controlling *C. parvum* than free chlorine alone at pH 6. Other studies have shown that chloramines generated using either pre- or post-ammoniation are adequate and, in some cases, superior to free chlorine in terms of indicator organism reductions (Norman et al. 1980; Selleck et al. 1978; Wolfe et al. 1985). The free chlorine pretreatment levels at pH 8 used in this study were demonstrated to give no reduction in infectivity. Sequential treatment produces a synergistic effect since increasing levels of free chlorine pretreatment reduce the subsequent monochloramine concentration and contact time necessary for a given level of inactivation. This synergism is explained if free chlorine pretreatment alters the permeability of the oocyst to monochloramine, allowing it to more readily diffuse through the oocyst wall.

The superior efficacy of ozone has made it one of the leading disinfectants for controlling protozoa in drinking water (Korich et al. 1990; Peeters et al. 1989; Wickramanayake et al. 1985). I.g.H. models were fitted to previously collected ozone disinfection data at 7° and 22°C (Finch et al. 1993). I.g.H. parameters m and n were estimated to have nearly the same value, less than unity, meaning that the ozone

inactivation kinetics of *C. parvum* is characterized by tailing-off and concentration and contact time are of equal importance. The fitted Hom-type rate law at 22°C was cross-validated using additional trials. The Arrhenius activation energy was calculated to be 3.8 kcal/mol, the magnitude of which suggests that inactivation of *C. parvum* by ozone is entirely controlled by mass transfer of ozone into the oocyst. I.g.H. models were used to develop process design requirements for 2 log-units inactivation of *C. parvum* by ozone at 7° and 22°C. For natural water contactor systems in which ozone residuals disappear rapidly, I.g.H. modeling confirms the design concept of applying higher ozone residuals for shorter contact times to maximize inactivation levels of *C. parvum*.

Free chlorine or monochloramine are frequently added as the final disinfectant in water treatment facilities that use ozone as the primary disinfectant. For pH 6 and 22°C free chlorine residuals typical of water distribution systems (<2.5 mg/L) and contact times approaching 1,000 min were found to provide nearly 4 log-units of *C. parvum* inactivation given an ozone pretreatment of 1.5 log-units inactivation. For free chlorine applied doses ranging from 0.5 to 2.5 mg/L, contact times >300 min and perhaps as high as 900 min were necessary in order that free chlorine exhibit synergism.

The efficacy of monochloramine to inactivate *C. parvum* oocysts at pH 8 and 22°C was enhanced by 0.6 to 1.9 log-units, independent of the ozone pretreatment level (0.6 to 2.4 log-units), for monochloramine CT values >100 mg·min/L. For a given level of ozone pretreatment, the level of *Cryptosporidium* inactivation by sequential exposure to <2.5 mg/L of monochloramine or free chlorine is about the same at pH 8 and 6, respectively. Ozone pretreatment enhances the efficacy of monochloramine to a greater extent than for

free chlorine since monochloramine is less effective than free chlorine at concentrations <5 mg/L. The use of ozone pretreatment followed by chlorine compounds gives water utilities an effective disinfection treatment for controlling waterborne *C. parvum*.

6.2 Conclusions

The major conclusions from this work can be summarized as follows:

1. The use of concentration × contact time (CT) tables by the SWTR may over or underestimate levels of inactivation because of the inadequacy of the Chick-Watson model and assumption of a constant disinfectant concentration.
2. The Incomplete gamma Hom (I.g.H.) is a robust inactivation kinetic model that accounts for disinfectant decay, describes the various types of microbial survival curves, and can be used to construct design charts providing initial disinfectant concentration and contact time settings required for a desired level of inactivation for regulatory or engineering design purposes.
3. Chemical disinfectant demand and decay kinetics of the source water needs to tested prior to engineering design of a disinfection system in order to determine the necessary applied dose that will provide the required initial disinfectant residual required.
4. The efficacy of free chlorine is dependent on pH conditions as hypochlorous acid is significantly more effective for inactivating *C. parvum* than is hypochlorite ion.
5. Free (available) chlorine at pH 6 and 22°C may provide a partial barrier (0.5 log-units inactivation) to *C. parvum* under practical treatment conditions.

6. Monochloramine at pH 8 and 22°C does have an effect on *C. parvum* but an apparent threshold concentration makes the use of monochloramine alone not feasible.
7. Chloramination at pH 8 and 22°C using post-ammoniation is more effective than free chlorine alone at pH 6 for controlling *C. parvum*.
8. For natural waters in which ozone residuals rapidly disappear ($k' \geq 0.2\ min^{-1}$), I.g.H. modeling confirms the design approach of applying higher ozone residuals for shorter contact times to maximize inactivation levels of *C. parvum*.
9. Inactivation of *C. parvum* by ozone is most likely controlled by mass transfer of ozone into the oocyst and the general rule that the reaction rate doubles for a 10°C rise in temperature is not applicable.
10. Ozone followed by free chlorine at pH 6 or monochloramine at pH 8 and 22°C offers a practical approach for controlling *C. parvum* in drinking water.

6.3 Recommendations

In order to control *C. parvum* in drinking water additional work needs to be undertaken to address the following issues:

1. The effect of pH, temperature, and chlorine to nitrogen weight ratio on the efficacy of chloramination practiced using post-ammoniation.
2. Confirm whether or not low levels of chlorine treatment lead to enhanced infectivity of *C. parvum* oocysts as discussed in Chapter 3. Conduct dose-response trials using CD-1 mice neonates and *C. parvum* oocysts exposed to

appropriate chlorine concentrations and contact times and compare to the dose-response model based on untreated *C. parvum* oocysts.

3. Validate the temperature coefficient, θ, of 1.023/°C to appropriately adjust the k parameter of the I.g.H. model at 1°C.
4. Conduct a few additional ozone disinfection trials at 22°C to augment the cross-validation data set to permit estimation of an independent set of I.g.H. model parameter estimates for comparison to the I.g.H. model at 22°C developed in this work.
5. For ozone followed by free chlorine or monochloramine, conduct additional experiments to study the influence of chlorine concentration and contact time on synergism and develop kinetic models.
6. Test the adequacy of the I.g.H. models developed in this work based on the Iowa strain of *C. parvum* to predict levels of inactivation for other strains of *C. parvum* oocysts suspended in laboratory water.
7. Test the adequacy of I.g.H. models developed in this work to predict levels of *C. parvum* oocyst inactivation observed in disinfection experiments involving natural waters representative of water treatment plant conditions.

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APPENDIX A

Parasite Methods

A.1 Production and Purification of *C. parvum* Oocysts

The strain of *C. parvum* oocysts that were used in this study comes from Dr. Frank W. Schaefer III (U.S. Environmental Protection Agency, Cincinnati, Ohio). Dr. Schaefer obtained the strain from Dr. Charles R. Sterling (Department of Veterinary Science, University of Arizona, Tucson, Arizona) who originally obtained this isolate from Dr. Harley Moon (National Animal Disease Center, Ames, Iowa). The oocysts used in this study were produced in male neonatal Holstein calves (*Bos taurus*) using a modification of the methods described elsewhere (Musial et al. 1987). Calves that are obtained at birth, were given up to 2 L of colostrum from a bottle. Within 12 h the animal was dosed from a bottle containing 2×10^7 *C. parvum* oocysts suspended in 2 L of milk replacer. The calves were maintained on a diet of 1 part milk replacer and 1 part electrolyte solution during fecal collection.

At the onset of scouring, the feces were collected in tap water for 3 or 4 days depending upon the intensity of the infection. The solution was sequentially passed through 10, 20, 60, 100, 200 and 400 mesh sieves (ASTM, West Conshohocken, PA) by agitating and washing the sieves with 0.01% (v/v) aqueous Tween 20. Concentration of the particulates from the sieved feces was by centrifugation at $1,100 \times g$ for 10 min.

The purification of oocysts for the experiments was done by concentrating the parasites using sucrose flotation and cesium chloride gradient ultracentrifugation. A 50 mL conical centrifuge tube was filled with 30 mL of 2 M sucrose solution onto which 5 to 9 mL of emulsified feces were layered. The tubes were mixed by inverting 10 times and centrifuged at $800 \times g$ for 10 min at 4°C. The oocysts found at the water-sucrose interface

were removed using a pipette and diluted 5 times using Milli-Q® water containing 0.01% Tween 20. The oocysts were then washed 3 times in Milli-Q® water containing 0.01% Tween 20 at 2,800 × *g* for 20 min at 4°C. After the final wash, the supernatant was removed and the oocyst pellet stored at 4°C prior to the final purification of the parasites using a cesium chloride gradient centrifugation (Kilani and Sekla 1987). The cesium chloride gradient was prepared in a 40 mL Beckman polyallomer ultracentrifuge tube. The gradient consisted of a bottom layer (9 mL of 1.4 g/mL cesium chloride), middle layer (9 mL of 1.1 g/mL cesium chloride) and a top layer (9 mL of 1.05 g/mL cesium chloride). Approximately 3 mL of the parasite material was gently layered on top of the cesium chloride gradient and centrifuged at 16,000 × *g* for 60 min using a swinging-bucket rotor (SW-28) at the slow brake setting (Beckman L7-55 ultracentrifuge). After centrifugation, the top dense band (out of three formed following ultracentrifugation) contains the purified oocysts which were removed using a pipette and placed in 15 mL polypropylene snap cap tubes. The tubes were filled with Milli-Q® water containing Tween 20 and washed twice by centrifugation at 14,500 × *g* for 10 min using a fixed-angle SS-34 rotor of a high speed centrifuge (Sorvall, RC5-B centrifuge). After the final washing step the oocysts were suspended in 0.01% Tween 20 containing 100 units penicillin per mL, 100 µg streptomycin per mL, and 100 µg gentamycin per mL and were stored at 4°C. Oocysts were used within 90 days of collection. *C. parvum* oocysts were never exposed to 2.5% potassium dichromate or sodium hypochlorite.

The oocyst concentration of the suspension was determined by counting the parasites using a hemocytometer. The typical concentration of oocysts in the stock suspension was 1.5 to 2. 5 × 10^8 per mL.

A.2 Infectivity in Outbred, Neonatal CD-1 Mice

A neonatal mouse model was used to evaluate infectivity of *C. parvum* (Ernest et al. 1986). Breeding pairs of outbred CD-1 mice were obtained from the Charles River Breeding Laboratories (St. Constant, Quebec, Canada). The animals were given food and water *ad libitum* and were housed in cages with covers fitted with a 0.22 µm filter in a specific pathogen-free (P-2 level) animal facility.

When required for a dose-response or disinfection experiment, an aliquot of the stock oocyst suspension was washed and concentrated by centrifugation. Since the absorbance of oocyst preparations at 254 nm was demonstrated to not differ for 2×, 4×, and 6× washings with Milli-Q® water, oocysts were prepared for experiments using 2× washings. Oocyst doses were prepared from the stock suspension of oocysts by serial dilution to obtain the required dose. The actual dose given to the mice was determined from a hemocytometer count of the stock suspension and the dilution factor. The mice were orally inoculated 4 days after birth using a micropipetter with a known number of oocysts suspended in 5 to 10 µL of Milli-Q® water.

The infectivity of the oocysts was determined 7 days after infection. The mice were killed by cervical dislocation and the lower half of the small intestine, the cecum and the colon removed and placed in 10 mL of Milli-Q® water. The intestines were homogenized for 10 s in a Sorvall Omni-Mixer and washed three times in Milli-Q® water containing

0.01% Tween 20 at 2,000 × *g* for 15 min. After centrifugation, the supernatant was discarded and 10 mL of Sheather's sugar solution was added to the pellet and centrifuged at 1,000 × *g* for 10 min. A few drops from the surface of the suspension were removed and examined with differential interference contrast (DIC) microscopy at 400 power. Mice were scored either positive or negative for oocysts (one or more oocysts) after examination of the slide.

A.3 *In vitro* Excystation

In vitro excystation was used in this study as a quality control procedure to monitor changes in the oocyst preparation over the duration of the experiments. The excystation procedure was modified after a previously reported method (Woodmansee 1987). An aliquot was removed from the stock oocyst suspension, washed once at 2,000 × *g* in Milli-Q® water and counted with a hemocytometer. Fifty thousand oocysts were placed in 0.5 mL PBS, and 0.5 mL of 2X excystation medium (0.05 g trypsin and 0.15 g sodium taurocholate in 5 mL of tissue culture grade PBS) was added. The oocysts were incubated in the excystation medium at 37°C in a water bath. Following 60 min in the water bath, the tubes were placed at room temperature for 30 min. The numbers of oocysts, shells and sporozoites were counted at 400× magnification using phase contrast microscopy. A minimum of 100 shells plus intact oocysts were counted for each trial. Preparations were kept on ice until the counts were done to reduce sporozoite mortality.

Excystation and theoretical sporozoite yield calculated by the Woodmansee (1987) method was determined by:

$$\text{Per cent excystation} = \frac{\text{shells}}{\text{intact oocysts} + \text{shells}} \times 100 \qquad \text{(A-1)}$$

The Woodmansee procedure is similar to that used for calculating excystation rates of *Giardia muris* (Schaefer 1990). The Korich *et al.* (1990) calculation is based on:

$$\text{Per cent excystation} = \left(\frac{S}{S + \text{intact oocysts}}\right) \times 100 \qquad \text{(A-2)}$$

where S is equal to the number of excysted sporozoites in the treated sample divided by the number of excysted sporozoites per shell in the control. Irrespective of the method used to calculate excystation, the inactivation was estimated from:

$$\log \frac{N}{N_o} = \log \left(\frac{\%\ \text{excystation}_{\text{treated}}}{\%\ \text{excystation}_{\text{control}}}\right) \qquad \text{(A-3)}$$

While no statistically significant difference ($p \leq 0.05$) was found between the Woodmansee (1987) and Korich et al. (1990) methods for the controls in our earlier study (Finch et al. 1994), the Korich et al. (1990) method produced consistent results when used after ozone disinfection. The Woodmansee (1987) calculation gave occasional anomalous results. For this reason, the Korich et al. (1990) procedure was used in this study.

A.4 Cyst Recovery and Animal Infection

After disinfection, oocysts suspended in 50 mL Nalgene® reactor/centrifuge tubes were recovered by centrifugation at 27,000 × *g* for 10 min at 4°C using an SS-34 rotor spun at 15,000 rpm in a Sorvall super speed RC2-B centrifuge. The contents of the 250 and 1,000 mL reactors were transferred to 250 mL Nalgene® polypropylene copolymer centrifugation tubes and recovered by centrifugation at 5,821 × *g* for 20 min at 4°C. Positive control samples concentrated using the two different centrifugation protocols showed no difference in infectivity or *in vitro* excystation. The supernatant was removed by aspiration leaving approximately 5 mL of concentrated sample containing the oocysts.

Oocysts were counted four times using a hemocytometer before removing about 5×10^4 oocysts for the excystation procedure. The remaining volume was concentrated further by centrifugation at 900 × *g* for 15 min. The supernatant was removed by aspiration leaving a 1 mL pellet that was transferred to a microcentrifuge tube. The original centrifuge tubes were washed with 0.01% Tween 20, saving the wash water. The wash water and pellet were centrifuged at 3,000 × *g* for 3 min in an MSE Microcentaur centrifuge (Johns Scientific, Inc.). The supernatant was removed from all of the ultracentrifuge tubes and the wash water pellet was resuspended in a small amount of 0.01% Tween 20, then added to the remaining oocysts, and centrifuged again at 3,000 × *g* for 3 min. The supernatant was removed and 1 mL of 0.01% Tween 20 was added to the tube. The oocysts were counted again, and when necessary, the solution was further concentrated by centrifugation. Serial dilutions were made and the results of four hemacytometer counts were tested against a Poisson distribution prior to administering a 5-10 µL inoculum to four day old CD-1 mice neonates. Two cohorts consisting of a minimum of five CD-1 neonates per cohort were used for each trial. The number of treated oocysts administered per neonate differed by a factor of ten between cohorts.

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APPENDIX B

Animal Infectivity Data for Chapter 3

Table B-1. Summary of *C. parvum* infection in neonatal CD-1 mice for each free chlorine trial in 0.05 M, pH 6.0, phosphate buffer

Trial No.	Inoculum oocysts, per animal	Live animals in cohort	Infected animals in cohort	Estimated infectious dose, oocysts per animal	Reduction in infectivity, log units	Inoculum oocysts, per control animal	Live animals in control group	Infected animals in control group	Estimated infectious dose, oocysts per animal	Reduction in control(s) infectivity, log units	Net reduction in infectivity, log units
2	2.36×10^{2}	5	0	< 6	> 1.61	3.50×10^{1}	5	5	> 49	< -0.15	> 1.76
	2.36×10^{3}	5	5	> 49	< 1.68	3.50×10^{1}	5	5	> 49	< -0.15	ID
12	2.00×10^{1}	5	1	6	0.54	1.90×10^{1}	5	3	23	-0.08	0.62
	4.20×10^{1}	5	2	12	0.53	1.90×10^{1}	5	3	23	-0.08	0.61
	8.30×10^{1}	5	3	23	0.56	1.90×10^{1}	5	3	23	-0.08	0.64
14	2.00×10^{1}	5	1	6	0.54	1.90×10^{1}	5	3	23	-0.08	0.62
	4.00×10^{1}	5	2	12	0.51	1.90×10^{1}	5	3	23	-0.08	0.59
	8.00×10^{1}	5	3	23	0.54	1.90×10^{1}	5	3	23	-0.08	0.62
120	5.20×10^{1}	5	1	14	0.58	5.10×10^{1}	5	2	45	-0.06	0.64
	5.19×10^{2}	5	3	121	0.63	5.10×10^{1}	5	2	45	-0.06	0.69
176	5.00×10^{2}	5	3	121	0.62	4.00×10^{2}	5	4	401	0	0.62
177	5.00×10^{2}	5	5	> 401	< 0.10	4.00×10^{2}	5	4	401	0	< 0.10
179	1.00×10^{2}	5	4	401	-0.60	4.00×10^{2}	5	4	401	0	-0.60
268	6.00×10^{3}	5	4	527	1.06	2.50×10^{2}	5	3	196	0.11	0.95
269	8.00×10^{2}	5	5	> 527	< 0.18	2.50×10^{2}	5	3	196	0.11	< 0.07
321	4.00×10^{2}	5	4	477	-0.08	3.60×10^{2}	5	5	> 477	< -0.12	> 0.04
323	4.00×10^{3}	5	5	> 477	< 0.92	3.60×10^{2}	5	5	> 477	< -0.12	< 0.92
351	4.00×10^{4}	5	0	< 41	> 2.99	2.00×10^{2}	5	3	199	0	> 2.99
519	1.50×10^{2}	4	2	69	0.34	5.00×10^{1}	2	1	69	-0.14	0.48

Table B-2. Summary of *C. parvum* infection in neonatal CD-1 mice for each free chlorine trial in 0.05 M, pH 8.0, phosphate buffer

Trial No.	Inoculum oocysts, per animal	Live animals in cohort	Infected animals in cohort	Estimated infectious dose, oocysts per animal	Reduction in infectivity, log units	Inoculum oocysts, per control animal	Live animals in control group	Infected animals in control group	Estimated infectious dose, oocysts per animal	Reduction in control(s) infectivity, log units	Net reduction in infectivity, log units
1	4.00×10^{1}	5	3	23	0.25	3.50×10^{1}	5	5	> 49	< -0.14	> 0.39
3	6.00×10^{1}	5	5	> 47	< 0.11	3.50×10^{1}	5	5	> 49	< -0.14	> 0.14
11	4.00×10^{1}	5	3	23	0.25	3.70×10^{1}	7	5	34	0.04	0.21
13	3.20×10^{1}	5	3	23	0.15	3.70×10^{1}	7	5	34	0.04	0.11
57	4.00×10^{1}	4	1	23	0.24	4.00×10^{1}	5	2	44	-0.04	0.28
121	4.40×10^{1}	5	1	14	0.51	4.10×10^{1}	5	1	14	0.48	0.03
152	3.90×10^{1}	5	5	> 401	< -1.01	4.10×10^{1}	5	2	45	-0.04	< -0.97
235	1.00×10^{3}	5	3	199	0.70	2.00×10^{2}	5	0	< 41	> 0.69	< 0.01
353	1.00×10^{2}	5	3	199	-0.30	2.00×10^{2}	5	3	199	0	-0.30
517	1.50×10^{3}	5	0	< 28	> 1.73	5.00×10^{1}	2	1	69	-0.14	> 1.87

Table B-3. Summary of *C. parvum* infection in neonatal CD-1 mice for each (3:1) preformed monochloramine trial in 0.05 M, pH 8.0, phosphate buffer

Trial No.	Inoculum oocysts, per animal	Live animals in cohort	Infected animals in cohort	Estimated infectious dose, oocysts per animal	Reduction in infectivity, log units	Inoculum oocysts, per control animal	Live animals in control group	Infected animals in control group	Estimated infectious dose, oocysts per animal	Reduction in control infectivity, log units	Net reduction in infectivity, log-units
32	1.00×10^{3}	5	1	6	2.21	2.00×10^{2}	5	3	23	0.94	1.27
77	6.00×10^{1}	5	1	18	0.54	6.00×10^{1}	5	1	18	0.54	0
79	1.00×10^{2}	5	0	< 18	> 0.76	6.00×10^{1}	5	1	18	0.54	> 0.22
	1.00×10^{3}	5	5	> 240	< 0.62	6.00×10^{1}	5	1	18	0.54	< 0.08
111	5.30×10^{1}	5	5	> 401	< -0.88	5.10×10^{1}	5	4	401	-0.90	< 0.02
114	4.69×10^{2}	5	1	14	1.54	5.10×10^{1}	5	4	401	-0.90	2.44
133	2.38×10^{3}	4	0	< 19	> 2.09	3.53×10^{2}	4	3	282	0.10	> 1.99
283	8.00×10^{2}	5	4	477	0.23	2.50×10^{2}	4	1	52	0.68	-0.45
285	8.00×10^{2}	5	0	< 41	> 1.30	2.50×10^{2}	4	1	52	0.68	> 0.62
	8.00×10^{3}	5	5	> 477	< 1.23	2.50×10^{2}	4	1	52	0.68	< 0.55
339	8.00×10^{2}	5	5	> 477	< 0.23	1.80×10^{2}	5	2	97	0.27	< -0.04
341	2.00×10^{2}	5	4	477	-0.38	1.80×10^{2}	5	2	97	0.27	-0.65
343	1.60×10^{5}	5	1	41	3.60	1.80×10^{2}	5	2	97	0.27	3.33
521	1.50×10^{2}	4	4	> 142	< 0.02	5.00×10^{1}	2	1	69	-0.14	< 0.16

Table B-4. Summary of *C. parvum* infection in neonatal CD-1 mice for a free chlorine pretreatment level of 4 to 11 mg·min/L in 0.05 M, pH 8.0, phosphate buffer

Trial No.	Inoculum oocysts, per animal	Live animals in cohort	Infected animals in cohort	Estimated infectious dose, oocysts per animal	Reduction in infectivity, log units	Inoculum oocysts, per control animal	Live animals in control group	Infected animals in control group	Estimated infectious dose, oocysts per animal	Reduction in control(s) infectivity, log-units	Net reduction in infectivity, log-units
184	8.60×10^{1}	5	1	14	0.80	1.00×10^{2}	5	2	45	0.35	0.45
	8.56×10^{2}	5	4	401	0.33	1.00×10^{2}	5	2	45	0.35	-0.02
185	3.99×10^{4}	5	3	121	2.52	1.00×10^{2}	5	2	45	0.35	2.17
190	4.00×10^{2}	5	4	401	0	1.00×10^{2}	5	3	121	-0.08	0.08
193	1.00×10^{3}	5	4	401	0.40	1.00×10^{2}	5	3	121	-0.08	0.48
274	5.00×10^{2}	5	5	> 527	< -0.02	2.50×10^{2}	5	3	196	0.11	< -0.13
276	2.50×10^{4}	5	5	> 527	< 1.68	2.50×10^{2}	5	3	196	0.11	< 1.57
365	1.80×10^{3}	4	1	119	1.18	1.80×10^{2}	5	2	234	-0.11	1.29
367	1.80×10^{2}	5	2	234	-0.11	1.80×10^{2}	5	2	234	-0.11	0
	1.80×10^{3}	5	4	1334	0.13	1.80×10^{2}	5	2	234	-0.11	0.24
369	1.80×10^{2}	5	2	234	-0.11	1.80×10^{2}	5	2	234	-0.11	0
	1.80×10^{3}	5	4	1334	0.13	1.80×10^{2}	5	2	234	-0.11	0.24

Table B-5. Summary of *C. parvum* infection in neonatal CD-1 mice for a free chlorine pretreatment level of 20 to 85 mg·min/L in 0.05 M, pH 8.0, phosphate buffer

Trial No.	Inoculum oocysts, per animal	Live animals in cohort	Infected animals in cohort	Estimated infectious dose, oocysts per animal	Reduction in infectivity, log units	Inoculum oocysts, per control animal	Live animals in control group	Infected animals in control group	Estimated infectious dose, oocysts per animal	Reduction in control(s) infectivity, log-units	Net reduction in infectivity, log-units
88	2.70×10^3	5	2	44	1.79	2.49×10^2	5	2	44	0.75	1.04
124	3.56×10^2	5	0	< 14	> 1.42	4.10×10^1	5	1	14	0.48	> 0.94
	3.56×10^3	5	5	> 401	< 0.95	4.10×10^1	5	1	14	0.48	< 0.47
132	2.41×10^3	4	4	> 282	< 0.93	3.53×10^2	4	3	282	0.10	< 0.83
155	4.25×10^2	5	5	> 401	< 0.03	4.10×10^1	5	2	45	-0.04	< 0.07
161	2.00×10^2	5	1	14	1.17	3.50×10^2	5	4	401	-0.06	1.23
192	5.00×10^2	5	2	45	1.05	1.00×10^2	5	3	121	-0.08	1.13
275	1.25×10^3	5	5	> 527	< 0.38	2.50×10^2	5	3	196	0.11	< 0.27
509	1.00×10^2	5	0	< 28	> 0.55	5.00×10^1	4	4	> 142	< -0.45	ID
	1.00×10^3	4	4	> 142	< 0.85	5.00×10^1	4	4	> 142	< -0.45	< 1.30
511	1.00×10^2	5	2	53	0.27	5.00×10^1	4	4	> 142	< -0.45	< 0.72
513	5.00×10^1	5	5	> 171	< -0.54	5.00×10^1	4	4	> 142	< -0.45	> -0.09
636	5.00×10^1	8	2	18	0.44	5.00×10^1	6	1	13	0.59	-0.15
638	5.00×10^1	7	3	32	0.19	5.00×10^1	6	1	13	0.59	-0.40
649	7.00×10^1	8	6	134	-0.28	7.00×10^1	10	9	297	-0.63	0.35
†651	7.00×10^1	5	2	45	0.19	7.00×10^1	10	9	297	-0.63	0.82
†653	7.00×10^2	5	4	165	0.63	7.00×10^1	10	9	297	-0.63	1.26
†655	7.00×10^2	10	2	22	1.50	7.00×10^1	10	9	297	-0.63	2.13
659	7.00×10^1	10	9	297	-0.63	7.00×10^1	10	9	297	-0.63	0
‡661	7.00×10^1	5	4	165	-0.37	7.00×10^1	10	9	297	-0.63	0.26
‡663	7.00×10^1	5	2	45	0.19	7.00×10^1	10	9	297	-0.63	0.82
‡665	7.00×10^2	10	8	165	0.63	7.00×10^1	10	9	297	-0.63	1.26

Note: ID - Indeterminate

†Trials serially correlated with trial 649

‡Trials serially correlated with trial 659

Table B-6. Summary of *C. parvum* infection in neonatal CD-1 mice for a free chlorine pretreatment level of 725 to 2,200 mg·min/L in 0.05 M, pH 8.0, phosphate buffer

Trial No.	Inoculum oocysts, per animal	Live animals in cohort	Infected animals in cohort	Estimated infectious dose, oocysts per animal	Reduction in infectivity, log units	Inoculum oocysts, per control animal	Live animals in control group	Infected animals in control group	Estimated infectious dose, oocysts per animal	Reduction in control(s) infectivity, log-units	Net reduction in infectivity, log-units
26	3.52×10^{3}	6	1	5	2.83	2.00×10^{1}	5	1	6	0.51	2.32
31	4.00×10^{4}	5	0	< 6	> 3.81	2.00×10^{2}	5	3	23	0.94	> 2.87
56	3.47×10^{4}	4	0	< 23	> 3.18	4.00×10^{1}	5	2	44	-0.04	> 3.22
125	4.59×10^{2}	5	3	121	0.58	4.10×10^{1}	5	1	14	0.48	0.10
154	3.47×10^{2}	5	3	121	0.46	4.10×10^{1}	5	2	45	-0.04	0.50
	3.47×10^{3}	5	4	401	0.94	4.10×10^{1}	5	2	45	-0.04	0.98
160	2.00×10^{3}	5	3	121	1.22	3.50×10^{2}	5	4	401	-0.06	1.28
163	7.00×10^{2}	5	3	121	0.76	3.50×10^{2}	5	4	401	-0.06	0.82
523	5.00×10^{1}	3	2	109	-0.34	5.00×10^{1}	2	1	69	-0.14	-0.20

APPENDIX C

Calculating The Ozone Decay Rate Constant

Appendix C summarizes the method that was used to estimate the ozone decay rate constant rate. First-order kinetics provided a reasonable approximation to describe the rate at which the concentration of aqueous ozone decreased in solution during the contact time.

The concentration of stock aqueous ozone was measured using ultraviolet spectrophotometry at 260 nm and a molar absorption coefficient of 3,300 $M^{-1}cm^{-1}$. A 1.0 cm quartz cuvette and model 601 Milton Roy spectrophotometer were used to take stock ozone measurements three times prior to and twice following addition of the calibrated volume of stock ozone solution to the reactor. The mean of these five stock ozone measurements was used to calculate the ozone dose applied to the reactor using a mass-calibrated Oxford pipette.

A diode-array spectrophotometer (Hewlett-Packard model 8452A) monitored the absorbance at 260 nm of the reactor solution. The reactor was continuously sampled in a closed loop at a flow rate of 8 mL/min. The sample was carried through a short piece of small diameter Teflon® tubing to a 35 µL flow cell with a light path of 1.0 cm. Ozone remaining at the end of the contact time was neutralized using 1 M sodium formate (BDH Inc., AnalaR grade). The absorbance profile of an HPC bacteria disinfection trial (first row of data in Table 2-2) is shown in Figure C-1.

The difference between the final baseline absorbance (following sodium formate addition) and measured absorbances over the contact time, multiplied by a concentration factor of 14.55, was used to calculate ozone residuals. The applied ozone dose is the mass of ozone delivered from the stock solution divided by the final volume of the

solution in the reactor. The initial ozone residual is the ozone concentration measured in the reactor immediately following addition of the stock solution. The instantaneous ozone demand is the difference between the calculated applied ozone dose and the measured initial ozone residual.

The rate at which ozone disappeared from the reactor, following any instantaneous ozone demand, was modeled using first-order kinetics. The ozone decay rate constant, k', for each trial was calculated using the Solver function of Microsoft® Excel® to regress equation 2-14 using the method of least-squares and a minimum of five data points, including initial and final measured residuals, from the absorbance profile (converted to concentrations). Absorbance values used for regression analysis were those points on the absorbance profile representative of the general trend of the curve, refer to Figure C-1. This regression approach is illustrated for *C. parvum* disinfection trials 210 and 221.

Trial 210

First-order decay rate, k', min-1 = 0.310
Residual Sum of Squares = 0.021

Time, seconds	Elapsed contact time, min	HP spec measured A260 nm	Observed O3 residual, mg/L	Predicted O3 residual, mg/L
13		0.01909		
27		0.01863		
39		0.09227		
42	0	0.09903	1.170	1.170
43	0.017	0.09807	1.162	1.164
54	0.200	0.08958	1.039	1.100
65	0.383	0.08351	0.950	1.039
85	0.717	0.07971	0.895	0.937
96	0.900	0.07449	0.819	0.885
120	1.300	0.07108	0.770	0.782
147	1.750	0.06435	0.672	0.681
150	1.800	0.06396	0.666	0.670
179	2.283	0.06020	0.611	0.577
192	2.500	0.05756	0.573	0.540
221	2.983	0.05096	0.477	0.465
236	3.233	0.04868	0.444	0.430
268	3.767	0.04485	0.388	0.365
281		0.01819	0.000	

Trial 221

First-order decay rate, k', min-1 = 0.459
Residual Sum of Squares = 0.002

Time, seconds	Elapsed contact time, min	HP spec measured A260 nm	Observed O3 residual, mg/L	Predicted O3 residual, mg/L
2		0.01282		
37		0.01308		
48		0.12279		
49	0	0.11412	1.472	1.472
72	0.383	0.09499	1.194	1.235
111	1.033	0.07759	0.941	0.916
150	1.683	0.05916	0.672	0.680
195	2.433	0.04671	0.491	0.482
279	3.833	0.02992	0.247	0.253
285	3.933	0.01295	0.000	

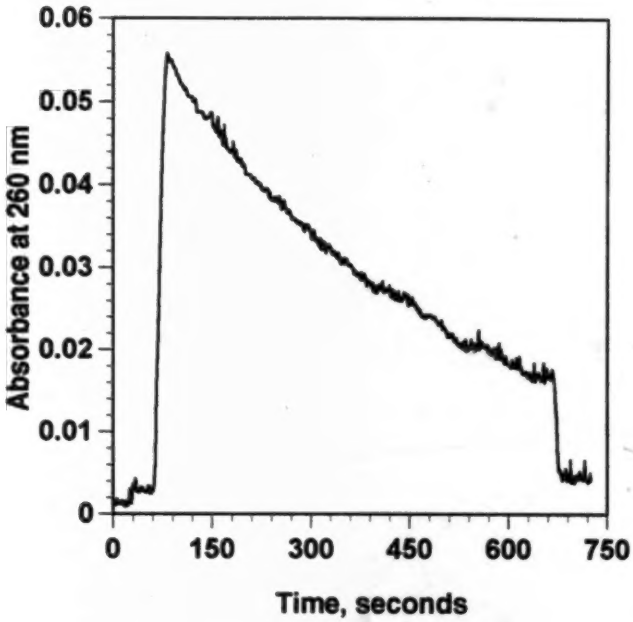

Figure C-1. Absorbance profile at 260 nm of reactor solution in which HPC bacteria is suspended in 0.05 M, phosphate buffer (first row of data in Table 2-2)

APPENDIX D

Curriculum Vitae

CURRICULUM VITAE

Lyndon L. Gyürék

PERSONAL:

Ph.D. Candidate
Department of Civil and Environmental Engineering
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Address:	9825 - 103 Street Suite 610 Edmonton, Alberta T5K 2M3
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EDUCATION:

1992 - present	Ph.D. Candidate in Environmental Engineering Department of Civil and Environmental Engineering University of Alberta Thesis topic: Ozone and Chlorine Inactivation of <u>Cryptosporidium</u> in Water Advisor: Dr. G.R. Finch Department of Civil and Environmental Engineering Co-advisor: Dr. M. Belosevic Department of Biological Sciences and Department of Medical Microbiology and Immunology
1994	Summer Professional Program Assessing Organic Pollutants in the Environment Massachusetts Institute of Technology
1992	B.Sc. in Civil Engineering (Co-operative Program) University of Alberta

PROFESSIONAL AFFILIATIONS:

Association of Professional Engineers, Geologists, and Geophysicists of Alberta
American Water Works Association
International Ozone Association
Alberta Writers Guild

AWARDS:

Natural Sciences and Engineering Res[illegible] il Postdoctoral Fellowship
Natural Sciences and Engineering Resea[illegible] l Postgraduate Scholarship
Walter H Johns Graduate Fellowship, Uni[illegible] Alberta

RESEARCH INTERESTS:
Water treatment disinfection, unit processes i[illegible] er and wastewater treatment, statistical methods of experimental design and analysis

PROFESSIONAL ACTIVITIES:

09/92 - present — Graduate Research and Teaching Assistant, Department of Civil and Environmental Engineering

- Responsible for collection and analysis of data involving disinfection studies funded by the American Water Works Research Foundation, U.S. Environmental Protection Agency, and Chemical Manufacturers Association
- Prepared and lectured material for a graduate course in the design and analysis of civil engineering experiments
- Supervised laboratory sessions for a number of undergraduate and graduate civil engineering courses

05/92 - 09/92 — Engineer, City of Edmonton Plants Engineering

- Prepared a report on the conceptual design of a sodium carbonate system for reducing water hardness at the Rossdale Water Treatment Plant
- Investigated the reliability of power backup systems at the Rossdale Water Treatment Plant

01/90 - 09/90 — Computer Programmer, Alberta Gas Transmission Division, Cost Estimating Group, Nova Corporation

- Assisted the Cost Estimating Department in the development of a mainframe program for estimating pipeline construction costs
- Prepared a report on the role of contingency in the estimate and a historical cost analysis of pipeline and meter station projects

PUBLICATIONS

Publications in refereed journals:

Belosevic, M., R.A. Guy, R. Taghi-Kilani, N.F. Neumann, L.L. Gyürék, L.R.J. Liyanage, P.J. Millard, and G.R. Finch. 1997. "Nucleic acid stains as indicators of *Cryptosporidium parvum* oocyst viability." *International Journal for Parasitology*, 27(7), 787-798.

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Engineering reports:

Bellamy, W., G.R. Finch, C.N. Haas, S. Shaw, L.L. Gyürék, and J. Oxenford. 1997. *The integrated disinfection design framework for potable water disinfection.* American Water Works Association and the AWWA Research Foundation: Denver, CO.

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